EVALUATION OF ANTIMICROBIAL AND ANTITUBERCULAR ACTIVITY OF *5-NITRO 2-THIOPHENE CARBOXALDEHYDE* BY *INVITRO* METHOD

A dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

CHENNAI - 600 032.

In the partial fulfillment of the requirements

for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

Reg No: 261226052

INSTITUTE OF PHARMACOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003.

APRIL – 2013-14

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF ANTIMICROBIAL AND ANTITUBERCULAR ACTIVITY OF *5-NITRO 2-THIOPHENE CORBOXALDEHYDE* BY *INVITRO* METHOD"** submitted by **Registration No. 261226052** in partial fulfillment of the requirements for the award of Degree of Master of Pharmacy in Pharmacology by the Tamilnadu Dr. M. G. R. Medical University, Chennai is a bonafide work done by her during the academic year 2013-2014.

The Dean,

Madras Medical College,

Chennai-600 003.

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF ANTIMICROBIAL AND ANTITUBERCULAR ACTIVITY OF 5-*NITRO 2- THIOPHENE CORBOXALDEHYDE* BY *INVITRO* METHOD"** submitted by **Registration No. 261226052** in partial fulfillment of the requirements for the award of Degree of Master of Pharmacy in Pharmacology by the Tamilnadu Dr. M. G. R. Medical University, Chennai is a bonafide work done by her during the academic year 2013-2014.

The Director and HOD,

Institute of Pharmacology,

Madras Medical College,

Chennai - 600 003.

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF ANTIMICROBIAL AND ANTITUBERCULAR ACTIVITY OF *5-NITRO 2-THIOPHENE CORBOXALDEHYDE* BY INVITRO METHOD"** submitted by Registration **No. 261226052** in partial fulfillment of the requirements for the award of Degree of Master of Pharmacy in Pharmacology by the Tamilnadu Dr. M. G. R. Medical University, Chennai is a bonafide work done by her during the academic year 2013-2014.

Mrs. M. Sakthi abirami, M.pharm.,

Tutor in Pharmacy,

Institute of Pharmacology,

Madras Medical College,

Chennai - 600 003.

ACKNOWLEDGEMENT

Though words are seldom sufficient to express my gratitude and feelings. It somehow gives an opportunity to thank those who helped me during the tenure of my study.

I would like to express my heartfelt gratitude to **Dean, Dr. R. VIMALA, M.D.,** Madras Medical College for permitting me to undertake the project during the period of my academic study.

First of all, I sincerely express my deep sense of gratitude to **Dr. R. Nandini, M.D.,** Director and HOD, Institute of Pharmacology, Madras Medical College, Chennai for her guidance, encouragement and optimistic approach with praise for the successful completion of my project work.

I express my respect and gratitude to **Dr. Jayalakshmi, M.D, D.T.C.D.,** Director and Professor, Institute of Microbiology, Madras Medical College, Chennai, for his valuable support.

I would like to express my heartfelt thanks and gratitude to my guide **Mrs. M. Sakthi abirami, M.Pharm.,** Tutor in Pharmacy, Madras Medical College, for her expert guidance and constant support during my dissertation work.

I owe my special thanks to **Dr. B. Kalaiselvi, M.D.,** Professor in institute of Pharmacology, Madras Medical College for her continuous support, Inspiration and valuable suggestions throughout my academic study.

My sincere thanks to **Dr. B. Vasanthi, M.D.,** Professor in Institute of Pharmacology, Madras Medical College for giving constant encouragement and support to complete my work successfully.

I would like to express my sincere thanks to **Dr. K. M. Sudha, M.D.,** Associate Professor in Institute of Pharmacology, Madras Medical College for her constant support throughout my study.

I am extremely thankful to **Dr. A. C. Yegneshwaran, M.D.,** (General Medicine), Tutor in Clinical Pharmacology, Madras Medical College, for his kind nature and support to complete my study.

I express my sincere thanks to **Dr. A. Suguna Bai, M.D.,** Assistant Professor in Institute of Pharmacology, Madras Medical College for her moral support and valuable suggestions during my study.

We express our deep sense of profound thanks to **Dr. Kishore bhat, Prof.&H.O.D.,** Department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum-590010 for given us report.

I express my sincere thanks to **Dr. K.G. Venkatesh, MD.,** Assistant Professor, Institute of Microbiology, Madras Medical college, Chennai, for his co-operation and encouragement and lending me all the facilities required to proceed with my study.

Heartfelt thanks to **Mrs. R. Indumathy, M.Pharm.,** Tutor in pharmacy and **Mrs. G. Sasikala, M.Sc.,M.Phil.,** Research Assistant in my department for their constant support and encouragement throughout the project.

I would like to extend my thanks to **Dr. Vijayarani, M.D., Dr. V. Chenthamarai, M.D., Dr. K. M. Malathi, M.D., Dr. Deepa, M.D.,** Assistant Professors in my department for their support during the study.

It's my immense pleasure to thank all the lab technicians of Institute of Pharmacology, Institute of Microbiology, Madras Medical College for their timely help.

Finally I am indebted to my parents, family members and my friends who have been solid pillars of everlasting support, encouragement, blessings that nobody can measure. There are no words to express it.

ABBREVATIONS

AFB	Acid –Alcohol Fast Bacilli
AIDS	Acquired Immune Syndrome
ARVs	Antiviral drugs
BCG	Bacillus of Calmette and Gurein
DOH	Department of Health
DOTS	Directly Observed Treatment Short Course
EMB	Ethambutol
HIV	Human Immuno deficiency Virus
INH	Isoniazid
MABA	Microplate Alamar Blue Assay
MDRTB	Multidrug-Resistant Tuberculosis
MOTT	Mycobacteria Other then Tuberculosis
MIC	Minimum Inhibitory Concentration
NTM	Non-Tuberculosis Mycobacteria
PZA	Pyrazinamide

PAS	Para Amino Salicyclic acid
RIF	Rifampicin
RNTCP	Revised National Tuberculosis Program
TB	Tuberculosis
WHO	World Health Organization
XDRTB	Extensively Drug Resistant Tuberculosis

INTRODUCTION

ANTIMICROBIALS:

Antimicrobial chemotherapy has been an important medical treatment. Since, the first investigations of antibacterial dyes by Ehrlich in the beginning of the twentieth century, however, by the late 1940's bacteria resistant to antimicrobials were recognized as a serious problem in clinical environments such as hospitals and care facilities.

Bacterial resistant forces the research communities to develop method of altering structures of antimicrobial compound to avoid their inactivation yet structural modification alone are not enough to avert bacterial resistance.

The increasing use of household antibacterial products and agricultural antibacterial fosters resistance to drug specific for human therapy and may have huge consequences for particularly children and elderly people.[(1)]

Bacterial cell walls are unique structures that serve as ideal targets for antimicrobial drugs. The agents that interfere with bacterial cell wall biosynthesis or cell integrity have been used therapeutically with high efficacy and good safety. Because there is no comparable structure in mammalians bacterial cell wall inhibitors can exhibits high target specificity with the side effect profiles that are target related, unlike some other classes of antibiotics. In addition, cell wall active agents are frequently bactericidal in their actions, providing the opportunity for complete bacterial clearance in serious infections.[(2)]

The multidrug resistant of bacterial and fungal strains of clinically important pathogens fetches the interest of scientist to develop newer broad spectrum antimicrobial agents.[(3)]

The less availability and high cost and greater side effects of new generation antibiotics necessiciates looking for the substances from alternative medicines which claimed antimicrobial activity have been reported in literature.[4-6]

ANTI-TUBERCULOSIS:

Tuberculosis (TB) is an infectious disease, caused by the bacterium called Mycobacterium tuberculosis which is slow growing bacteria. It was first isolated by Robert Koch in 1882 (Rathore madhu et al., 2012).[7] Tuberculosis remains a global public health problem especially in developing countries. It is an air born communicable disease caused by transmission of aerosolized droplets of Mycobacterium tuberculosis, affecting all the organs in the body that rich in blood and oxygen, the lungs being most commonly affected.

Despite the availability of effective anti-tuberculosis chemotherapy for over 60 years, the incidence of tuberculosis continues to persist. The World Health Organization (WHO) declared tuberculosis as global emergence in 1993.

In 2010, there were an estimated 8.8 million incident cases of tuberculosis globally, equivalent to 128 cases per 1, 00,000 population. Many of the reported number of cases in 2010 occurred in Asia (59%) and Africa (26%). The five countries with the largest number of tuberculosis cases during 2010 were in **India** (2.0 million – 2.5 million), china (0.9 million – 1.2 million), South Africa (0.40 million – 0.59 million), Indonesia (0.37 million – 0.54 million) and Pakistan (0.33 million – 0.48 million). India alone accounted for an estimated one quarter (26%) of all tuberculosis cases worldwide, and China and India together accounted for 38% (WHO Report 2011).[8]

World Health Organization and National TB Programme recommend four drugs (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol) for 2 months in initial phase and then two drugs (Rifampicin and Isoniazid) for 4 months as continuation phase via DOTS (Directly Observed Therapy Short course) for current treatment of tuberculosis **(TB)**. The regimen must be administered for at least 6 months to be fully effective in humans.[9,10].

A number of antimicrobial agents already exist but the search for new drugs continues since the target organism evolves into new genetic variant. The chemical efficacy of many existing anti-biotic is being threatened by the emergence of multidrug resistance pathogens. The increasing incidence of MDR (Multi Drug Resistance) and XDR (Extreme Drug Resistance)-Tuberculosis worldwide highlights the urgent need to search for anti-tuberculosis compound/drug, to increasing the probability of finding appropriate drug. The present study was carried out to investigate and estimate the new compound which have been used to treat respiratory infections for its anti-tubercular activity by using H37Rv standard strain and resistant isolates.[11]

The original antibiotics were derived from fungal source. There can be referred to as natural antibiotics.

- Organisms develop resistance faster to the natural antimicrobials because they have been pre-exposed to these compounds in nature. Natural antibiotics are often more toxic than synthetic antibiotics.
- Semi synthetic drug were developed to decrease the toxicity and increase the effectiveness.
- Synthetic drug have an advantage that the bacteria are not exposed to the compound until they are released. They are also designed to have even greater effectiveness and less toxicity.

5-Nitro 2-Thiophene carboxaldehyde is a synthetic compound which is used as intermediate compound to synthesis new derivatives of many novel compounds having excellent antimicrobial, antitubercular, anti-inflammatory, anticancer, analgesic, antihistaminic, antiviral, antipsychotic, diuretic activity. In this study antimicrobial and anti-tubercular activity of *5-Nitro 2-Thiophene carboxaldehyde* compound has been screened.

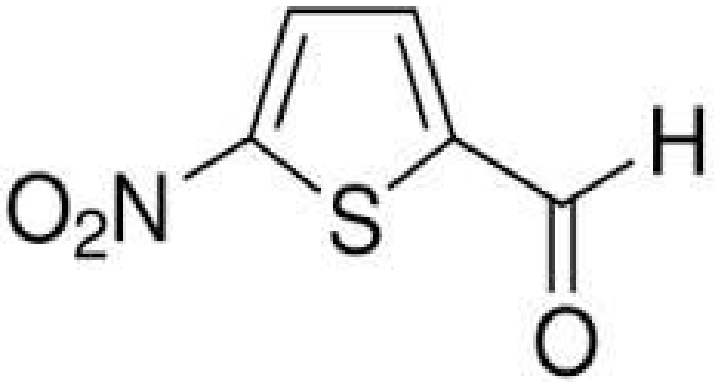
AIM AND OBJECTIVES

- To evaluate the antibacterial activity of *5-Nitro 2-thiophene carboxaldehyde* compound by Agar dilution and Agar diffusion method.

- To evaluate the antifungal activity of *5-Nitro 2-thiophene carboxaldehyde* compound by Agar dilution method.

- To evaluate the anti-tubercular activity of *5-Nitro 2-thiophene carboxaldehyde* compound by Microplate Alamar Blue Assay method (MABA) using **H37Rv** tubercular cell lines.

COMPOUND PROFILE



Molecular Formula : C5H3NO3S

Molecular Weight :157.15 gm/mol.

APPEARANCE:

Colour : Yellow to Brown

Form : Solid (Crystals, Fibres, crystalline powder)

Melting Point : 75 - 77ºC – lit.

Purity : > 97.5%

Solubility 10mg/ml (1%), Acetone : Clear

Solubility Colour : Colourless to Yellow colour

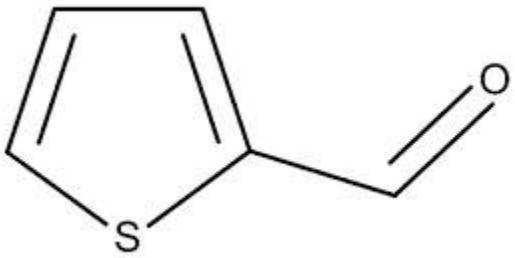
Incompatible Material : Strong oxidizing agent, strong base

5-NITRO 2-THIOPHENE CARBOXALDEYDE

Description:

5-Nitro 2-Thiophene carboxaldehyde is an aromatic heterocyclic compound consisting of four carbon atoms and one sulfur atom in a five membered ring with Nitro group at the 5th position. Thiophene was discovered by Victor Meyer in 1883 as a contaminant in benzene.(12)

Thiophene was observed that isatin forms a blue dye, if it is mixed with sulfuric acid and crude benzene. Victor Mayer was able to isolate the substance responsible for this reaction from benzene. This newly heterocyclic compound was thiophene.(13)



Thiophenes are important class of heterocyclic compounds and are recurring building blocks in organic chemistry with applications in pharmaceutical. The benzene ring of a biologically active compound may often be replaced by a thiophene without loss of activity.(14) Thiophene and its derivatives occur in petroleum, sometimes in concentration upto 1-3 %. The thiophenic content of liquids from oil and coal is removed via the hydrodesulphurization (HDS) process.

Therapeutic importance:

In Medicinal chemistry, Thiophene derivatives have been well known for their therapeutic applications. Many thiophene derivatives have been developed as chemotherapeutic agents and are widely used. Thiophene nucleus is one of the most important heterocycles exhibiting remarkable pharmacological activities.

Over the recent years there has been an increasing interest in the medical field of thiophene because of their biological significance.

1. Analgesic[15]
2. Antimalarial[16]
3. Anticonvulsant[17]
4. Antifungal[18]
5. Antihistaminic[19]
6. Anti-inflammatory[20]
7. Antitumor[21]
8. Antiviral[22]
9. Diuretic[23]
10. Insecticidal[24]
11. Antipsychotic[25]

In the June 16, 2013, online edition of the *Journal Chemical Biology* revealed that treatment of Mycobacterium tuberculosis with an experimental thiophene drug was bactericidal and equivalent to treatment with first line drug isoniazid, but was less likely to permit emergent resistant, combined isoniazid and thiophene treatment resulted in complete inhibition of Mycobacterium tuberculosis growth.

REVIEW OF LITRATURE:

Anti-inflammatory activity:

Sahar Mohamed Ibrahim BADR, *et al.,* Synthesis and anti-inflammatory activity of novel 2,5-disubstituted thiophene derivatives were studied. New series of **2,5-disubstituted thiophenes** were synthesized. Thiosemicarbazones 1a-b were reacted with various reagents, such as diethyl-2-bromomalonate, ethyl-2-chloroacetoacetate, thioglycolic acid, 4-substituted phenacyl bromides, and acetic anhydride, to afford heterocyclic substituted thiophene derivatives 2a-b, 3a-b, 4a-b, 5a-b, 6a-b, and 7a-b, respectively. Moreover, cyclization of the key intermediate 1b with chloroacetic acid yielded thiazolidine 9, which on reaction with appropriate aromatic aldehydes afforded the corresponding arylidene derivatives 10a-f. Finally, reaction of N – arylidene cyanoacetohydrazide 11 with sulfur and phenyl isothiocyanate yielded thiazoline derivative 12, which on treatment with tri ethyl ortho formate and acetic anhydride afforded thiazolo (4,5-d) pyrimidinone derivative 13. Some of the newly synthesized compounds showed promising **anti-inflammatory activity**.

Antimalarial activity:

Tedlouti *et al.,* Evaluation of the antimalarial activity of new compound against Plasmodium falsiparum *invitro* and Plasmodium berghei *invivo*, on mice experimentally. These hydrazones were obtained by condensation of appropriate hydroxines with thiophene 2-carboxaldehyde, thiophene 2-carboxaldehyde, ***5-Nitro thiophene 2-carboxaldehyde***. The compound of series 3,5-nitrothiophene 2-carboxaldehyde presented significant effects both *invitro* and *invivo* methods.

Anti-Inflammatory and analgesic activity:

Sondhi, S.M. *et al.,* Synthesis of some thiophene, imidazole and pyridine derivatives exhibiting good anti-inflammatory and analgesic activities were studied. A series of thiophene

derivatives 1a-d and 2a-c were synthesized by condensation of ***5-Nitro 2-Thiophene carboxaldehyde*** with mono and diamine respectively. Compound 1b and 2c exhibited good anti-inflammatory activity.

Antimicrobial and antitumor activity:

Etaiw S.E. ***et al.,*** Synthesis, spectral, antimicrobial and antitumor assessment of Schiff base derived from 2-amino benzthiazole and its transition metal complexes were studied. N-(thiophene-2-yl methylene) benzo [d] thiazole-2-amine Schiff base (L) derived from 2-amino benzthiazole and 2-thiophene carboxaldehyde and its complexes with Cu(II), Ni(II), and Zn(II) were prepared. In view of the biological activity of the Schiff base and its complexes, it has been observed that the antimicrobial activity of the Schiff base increased on complexation with the metal ions and having significant *invitro* antitumor activity.

Anticonvulsant and antiparkinsonism activity:

Abdel-Galil E. Amr. ***et al.,*** Synthesis and reactions of some fused oxazinone, pyridinone, thiopyrimidone and triazone derivatives with a thiophene ring to offerd the corresponding thioglycolic acid pyrimidone derivatives. The pharmacological screening showed that many of these obtained compounds have good **analgesic, anticonvulsant and anti-parkinsonism activities** comparable voltarene, carbamazepine and benzotropine as reference drugs.

Antitubercular activity:

Joshi H.S. ***et al.,*** Synthesis of some New Thiosemicarbazide and 1, 3, 4-Thiadiazole Heterocycles bearing [b] Thiophene nucleus as a potent Antitubercular and Antimicrobial Agents were studied. Reaction of 2-hydrazinocarbonyl-3-chloro-5phenoxy-benzo[b] thiophene with different

substituted phenyl isothiocyanate gave N-substituted arylthiosemicarbazide derivatives (1a-h). 1,3,4-Thiadiazole derivatives (2a-h) were prepared by the cyclization of arylthiosemicarbazides (1a-h) with concentrated sulphuric acid. All the compounds were screened for their **antitubercular activity against Mycobacterium tuberculosis (H37Rv) and antimicrobial activity** against various microorganisms.

ANTI MICROBIAL ACTIVITY

ANTIBACTERIAL ACTIVITY:

The science dealing with the study of the prevention and treatment of disease caused by micro-organisms is known as medical microbiology. Its sub disciplines are virology (study of viruses), bacteriology (study of bacteria), mycology (study of fungi), phycology (study of algae) and protozology (study of protozoa). For the treatment of diseases inhibitory chemicals employed to kill micro-organisms or prevent their growth, are called antimicrobials agents. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders. The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses), bactericides (killing bacteria), algicides (killing algae) or fungicides (killing fungi).

Paul Ehrlich used the term chemotherapy for curing the infectious disease without injury to the host's tissue, known as chemotherapeutic agents such as antibacterial, antiprotosoal, antiviral, antineoplastic, antitubercular and antifungal agents. Later on, Domagk (1953) prepared an important chemotherapeutic agent sulfanilamide.

BACTERIA:

The bacteria are microscope organisms with relatively simple and primitive forms of prokaryotic type. Danish Physician Christian Grams, discovered the differential staining technique known as Gram staining, which differentiates the bacteria into two groups "Gram positive" and "Gram negative", Gram positive bacteria retains the crystal violet and resist decolorization with

acetone or alcohol and hence appear deep violet in colour; while Gram negative bacteria, which loose the crystal violet, are counter-stained by saffranin and hence appear red in color.[26]

These two groups of bacteria are recently classified into four different categories as follows:

(1) The world of bacteria I : "Ordinary" Gram negative bacteria
(2) The world of bacteria II : "Ordinary" Gram positive bacteria
(3) The world of bacteria III : "Bacteria" with unusual properties
(4) The world of bacteria IV : Gram positive filamentous bacteria of complex morphology.

CLASSIFICATION OF ANTIBACTERIAL AGNETS

The antibacterial agents are classified in three categories:

I. Antibiotics chemotherapeutic agents.
II. Non-antibiotic chemotherapeutic agents
(Disinfectants, antiseptics and preservatives
III. Immunological products.

ANTIBIOTICS:

They are produced by micro-organisms or they might be fully or partly prepared by chemical synthesis. They inhibit the growth of micro-organisms in minimal concentrations. Antibiotics may be of microbial origin or purely synthetic or semi synthetic.[27] They can be classified by manner of mechanisms of action.

CLASSIFICATION OF ANTIBIOTICS ACCORDING TO THEIR MECHANISMS OF ACTION :(Berdy, 1974).[28] Table - 1

S.NO	MECHANISM OF ACTION	EXAMPLE
1	Inhibit cell wall synthesis	Penicillins, Cephalosporins, Cycloserine, Vancomycin, Bacitracin
2	Cause leakage form cell membranes	Polymyxins, Colistin, Bacitracin, Amphotericin-B, Nystatin, Hamycin
3	Inhibit protein synthesis	Tetracyclins, Chloramphenical, Erythromycin, Clindamycin, Linezolid.
4	Cause misreading of mRNA code and affect permeability	Aminoglycosides: Streptomycin, Gentamycin, Amikacin, etc
5	Inhibit DNA gyrase	Fluoroquinolones: Ciprofloxacin, Ofloxacin Norfloxacin, Pefloxacin, etc
6	Interfere with DNA function	Rifampicin, Metronidazole
7	Interfere with DNA synthesis	Idoxuridine, Acyclovir, Zidovudine
8	Interfere with intermediary metabolism	Sulfonamides, Sulfones, PAS, Trimethoprim, Pyrimethamine, Ethambutol

Synthetic antimicrobial agents include sulfonamides, Diamino pyrimidine derivatives, antitubercular compounds, nitrofuran compounds, 4-quinoline antibacterials, imidazole derivatives, flucytosine etc.

(II) NON-ANTIBIOTICS CHEMOTHERAPEUTIC AGENTS:

The second category of antibacterial agents includes non-antibiotic chemotherapeutic agents which are as follows:

1. **Acids and their derivatives**

   Some organic acids such as ascorbic, benzoic, lactic and propionic acids are used for preserving food and pharmaceuticals. Salicylic acid has strong antiseptic and germicidal properties as it is a carboxylated phenol. The presence of –COOH group appears to enhance the antiseptic property and to decrease the destructive effect. Benzoic acid is used externally as an antiseptic and is employed in lotion and ointment. Benzoic acid and salicylic acid are used to control fungi that cause disease such as athlete's foot. Benzoic acid and sodium benzoate are used as antifungal preservatives. Mandolic acid possesses good bacteriostatic and bactericidal properties.

2. **Alcohols and related compounds**

   They are bactericidal and fungicidal, but are not effective against endospores and some viruses. Various alcohols and their derivatives have been used as antiseptics e.g. ethanol and propanol. The antibacterial value of straight chain alcohols increases with an increase in the molecular weight and beyond C8- the activity begins to fall off. The isomeric alcohol shows a drop in activity from primary, secondary to tertiary. Ethanol has extremely numerous uses in Pharmacy.

3. **Chlorination and compound containing chlorine:**

   Chlorination is extensively used to disinfect drinking water, swimming pools and

for the treatment of effluent from industries. Robert Koch in 1981 first referred to the bactericidal properties of hypochlorites. N-chloro compounds are represented by amides, imides and amidines wherein one or more hydrogen atoms are replaced by chlorine.

4. **Iodine containing compounds:**

Iodine containing compounds are widely used as antiseptic, fungicide and amoebicide. Iodophores are used as disinfectants and antiseptics. The soaps used for surgical scrubs often contain iodophores.

5. **Heavy metals:**

Heavy metals such as silver, copper, mercury and zinc have antimicrobial properties and are used in disinfectant and antiseptic formulations. Mercurochrome and Merthiolate are applied to skin after minor wounds. Zinc is used in antifungal antiseptics. Copper sulfate is used as algicides.

6. **Oxidizing agents:**

Their value as antiseptics depends on the liberation of oxygen and all are organic compounds.

7. **Dyes:**

Organic dyes have been extensively used as antibacterial agents. Their medical significance was first recognized by Churchman[(29)] in 1912. He reported inhibitory effect of Crystal violet on Gram-positive organism. The acridines exert bactericidal and bacteriostatic action against both Gram-positive and Gram-negative organisms.

8. 8-Hydroxyquinolines:

8-Hydroxyquinolines or oxine is unique among the isomeric hydroxyl-quinolines, for it alone exhibits antimicrobial activity. This attributes to its ability to chelate metals,[30] which the other isomers do not exhibit.

9. Surface active agents:

Soaps and detergents are used to remove microbes mechanically from the skin surface. Anionic detergents remove microbes mechanically; Cationic detergents have antimicrobial activities and can be used as disinfectants and antiseptics.

(III) IMMUNOLOGICAL PRODUCTS:

Certain immunological products such as vaccines and monoclonal antibodies are used to control the diseases as a prophylactic measure.

Mode of action:

Antimicrobial drugs interfere chemically with the synthesis of function of vital components of microorganisms, the cellular structure and functions of eukaryotic cells of the human body. These differences provide us with selective toxicity of chemotherapeutic agents against bacteria.

Antimicrobial drugs may either kill microorganisms outright or simply prevent their growth. There are various ways in which these agents exhibit their antimicrobial activity.[31] They may inhibit

1. Cell-wall synthesis
2. Protein synthesis
3. Nucleic acid synthesis
4. Enzymatic activity

5. Folate metabolism or
6. Damage cytoplasmic membrane

Bacteriostatic dyes:

Stearn and Stearn[32] attributed the bacteriostatic activity to triphenylmethane dyes. Fischer and Munzo[33] have found the relationship between their structure and effectiveness of such dyes.

A number of drugs are metal-binding agents. The chelates are the active form of drugs. The site of action within the cell or on the cell surface has not been estabilished. The site of action of oxine and its analogs has been suggested inside the bacterial cell[34] or on cell surface.[35]

Detoxification of antibacterials:

p-Aminobenzoic acid is a growth factor for certain micro-organisms and competitively inhibits the bacteriostatic action of sulfonamides. The metabolites identified in man are p-amino-benzoylglucoronide; p-aminohippuric acid, p-acetylaminobenzoic acid. 8-Hydroxyquinoline (oxine) and 4-hydroxyquinoline are excerted as sulfate esters or glucorinides.

ANTIFUNGAL ACTIVITY:

There are perhaps over 10,000 species of fungi, but less than 100 cause diseases in human.[36] Fungi may cause benign, but unsightly infections of the skin, nail or hair, relatively trivial infection of mucous membranes (thrush) or systemic infection causing progressive often fatal disease.

CLASSIFICTION OF MEDICALLY IMPORTANT FUNGI:[37]

1. True yeasts (e.g. Cryptococcus neoformans)
2. Yeast like fungi that produce a pseudomycelium (e.g. Candida albicans)

3. Filamentous fungi that produce a true mycelium (e.g. Aspergillus fumigatus)
4. Dimorphic fungi that grow as yeast or filamentous fungi depending on the cultural conditions (e.g. Histoplasma capsulatum)

ANTIBIOTIC RESISTANCE:

Antibiotics are extremely important in medicine, but unfortunately bacteria are capable of developing resistance to them. Antibiotic-resistance bacteria are germs that are not killed by commonly used antibiotics. When bacteria are exposed to the same antibiotics over and over, the bacteria can change and are no longer affected by the drug. Bacteria have number of ways how they become antibiotic resistance. For example, they possess an internal mechanism of changing their structure, so the antibiotic no longer works, they develop ways to inactivate or neutralize the antibiotic. Also bacteria can transfer the genes coding for antibiotic resistance between them, making it possible for bacteria never exposed to an antibiotic to acquire resistance from those which have. The problem of antibiotic resistance is worsened when antibiotics are used to treat disorders in which they have no efficacy (e.g. antibiotics are not effective against infections caused by viruses), and when they are used widely as prophylaxis rather than treatment. Resistance to antibiotics possesses a serious and growing problem, because some infectious diseases are becoming more difficult to treat. Resistant bacteria do not respond to the antibiotics and continue to cause infection. Some of these resistant bacteria can be treated with more powerful medicines, but there some infections that are difficult to cure even with new or experimental drugs.[38]

The incidence of multidrug-resistant pathogenic bacteria is increasing. On additional reason for developing new antibiotics is related to their own toxicity. As with other therapeutic agents, the use of antibiotics may also cause side effects in patients. These include mild reactions such as

stomach upset, vomiting and diarrhoea (cephalosporins, macrolides, penicillins and tetracyclines), rash, other mild and severe allergic reactions (cephalosporins and penicillins), sensitivity to sunlight (tetracyclines), nervousness tremors and seizures (quinolones). Some side effects are more severe not depending on the antibiotic may disrupt the hearing function (aminoglycosides), kidneys (aminoglycosides and polypeptides) or liver (rifampin). To counteract the resistance by microbes there is a need to invent new drugs, which are more safe and effective.

In order to combat the microbial infections anti-microbial agents are frequently used concurrently i.e the combined use of antimicrobials with following objectives,

- To prevent emergence of resistance
- To lower the adverse or side effects
- To have a synergistic effect
- To broaden the antimicrobial spectrum

Since micro-organisms develop rapid resistance there is an ample scope for the development of antibacterial agents that are active against resistant bacteria.[39]

5-nitro-2-thiophene carboxaldehyde is reported to have antimicrobial activity. Hence it was proposed to study the antimicrobial activity against 5 bacterial organisms and 2 fungal organisms which are as follows.

Antibacterial activity was carried against 5 organisms:

1. Staphylococcus aureus (Gram positive)
2. Enterococcus(Gram positive)
3. Escherichia coli(Gram negative)

4. Pseudomonas aeruginosa(Gram negative)
5. Salmonella Typhi(Gram negative)

Antifungal activity was carried against 2 organisms:

1. Candida albicans
2. Aspergillus flavus

Staphylococcus aureus

It is Gram-positive cocci, ovoid or spheroidal, non-motile, arranged in group of clusters; they grow on nutrient agar and produce colonies, which are golden yellow, white or lemon yellow in colour. Pathogenic strains produce, coagulated and ferment glucose lactose, mannitol with production acid, liquefy gelation and produce pus in the lesion.[40]

Infections:

1. Septic arthritis
2. Endocartitis
3. Pneumonia
4. Atopic dermatitis[41]

Enterococcus:

It is a Gram-positive cocci that occur in short chain which is facultative anaerobic organisms (i.e) they are capable of cellular respiration in both oxygen-rich and oxygen-poor environments.[42] They are tolerant to wide range of environmental condition.[28]

Infections:

1. Urinary tract infection

2. Bacteremia
3. Bacterial endocarditis
4. Diverticculitis and meningitis[43]

Escherichia coli:

They are Gram-negative rods, motile with peritrichate flagella or non motile. They do not form spores. All are commonly found in intestine of warm blooded organisms. It causes food contamination. It is used for producing the vitamin K2, preventing the estabilishment of pathogenic bacteria, used as plasmid and restriction enzyme to create Recombinant DNA and which is ideal organisms to test environmental sample for fecal contamination.

Infections:

1. Gastroenteritis
2. Urinary tract infection
3. Neonatal meningitis
4. Hemolytic uremic syndrome
5. Peritonitis
6. Mastitis
7. Septicemia
8. Pneumonia[44]

Pseudomonas aureginosa:

They are Gram-negative, aerobic short rod shape coccobacilli with unipolar motility. Organisms are non-sporulating and non-capsulated, however, few strains possess slime layer of up of

polysaccharides. It is identified as pearlescence appearance and grap like or tortilla like odour *invitro* studies. It is usually found widely in the environment such as soil, water and plants. It mainly causes nasocomical infections.

Infections:

1. Pneumonia
2. Meningitis
3. Endocartitis
4. Osteomyelitis
5. Gastrointestinal tract infections(Diarrhoea, enteritis, enterocolitis)
6. Skin and soft tissue infections (ecthyma gangrenosum)

Salmonalla typhi:

Salmonalla is a Gram-negative, rod-shaped, non-spore forming, motile enterobacteria, facultative anaerobes belong to the family of enterobacteriacea, which is susceptible to various antibiotics.

Infections:

1. Typhoid fever
2. Diarrhoea[(45)]

Candida albicans:

Candida species reproduce by yeast like budding cells but they also show formation of pseudomycellum. These pseudomycellum are chains of elongated cells formed from buds and the buds elongated without breaking of the mother cell. They are very fragile and separate easily.

Mycelia also form by the elongation of the germ tube by a mother cell. It may remain as a commensal of the mucous membrane with or without causing any pathogenic changes to the deeper tissues of the same fungus may cause pathological lesion of the skin. Such a fungus under favorable conditions can cause superficial, intermediate of deep mycoses depending on the condition of the host.

Infections:

1. Oral candidiasis, oropharyngeal candidiasis
2. Vulvovaginal yeast infection
3. Diaper rash
4. Candidemia[46]

Aspergillus flavus:

The Aspergilclli are widespread in nature, being found in fruits, vegetables and other substances, which may provide nutrient. Some species are involved in food spoilage. They are important economically because they are used in a number of industrial fermentations, including the production of citric acid and gluconic acid. Aspergilli grow in high concentrations of sugar and salt, indicating that they can extract water required for their growth from relatively dry substances.[47]

ANTITUBERCULOSIS:

MYCOBACTERIA

The genus Mycobacterium belongs to the order Actinomycetales and the family Mycobacteriaceae; it is characterized by non-motile, non-sporulating rods that resist decolorization with acidified organic solvents and alcohol. For this reason, they are also called acid-fast bacteria.

Some mycobacterial species are pathogenic for humans: they so called Mycobacterium complex includes M.tuberculosis, M. bovis, and M. africanum. Mycobacterium macroti is pathogenic for voles, field mice, and other rodents, but it was considered nonpathogenic in humans. However, in recent years, it has been demonstrated that it can cause disease in immune-suppressed, and particularly, in HIV positive patients. Mycobacterium leprae, pathogenic for man, was considered of uncertain taxonomy for some time, but now is definitely classified among Mycobacteria. The study of its genome has contributed to clarify its position in this genus. The development of recombinant DNA technology has allowed us to define better the species belonging to these groups, but the general definition still does not seem satisfactory. A common definition in the recent years has been "Mycobacteria other than tuberculosis" (MOTT), but now "Non-Tuberculous Mycobacteria" (NTM) is preferred.

The most recent and appropriate grouping of these organisms, proposed by the American Thoracic Society, is based on the type of clinical disease they produce: Pulmonary disease, lymphadenitis, cutaneous disease, and disseminated disease. The term mycobacteriosis is proposed for the diseases caused by these organisms.[48]

PATHOGENESIS AND EPIDEMIOLOGY

Tuberculosis is sometimes an acute but more frequently a chronic communicable disease that derives its character from several properties of the tubercle bacillus, which in contrast with many common bacterial pathogens, multiplies slowly, does not produce exotoxins, and does not stimulate an early reaction from the host. The tubercle bacillus is also an intracellular parasite, living and multiplying inside macrophages.

Chronic pulmonary tuberculosis in adults may be due to reactivation of the primary infection or to exogenous re-infection. A typical characteristic of tuberculosis is the formation in the infected tissue nodular formations called tubercles, which can have different sizes and different modes of diffusion, giving rise to various clinical forms called military, infiltrate, lobar tuberculosis, and so on. The disease progresses by means of ulceration, cessation and cavitations, with bronchogenic spread of infectious material. Healing may occur at any stage of the disease by processes of resolution, fibrosis, and calcification.

Control of the disease has been achieved in part through mass vaccination with BCG (Bacillus of Calmette and Guerin, an attenuated strain of M. tuberculosis bovis).[49]

Figure - 1

Mycobacterium Tuberculosis Cell structure

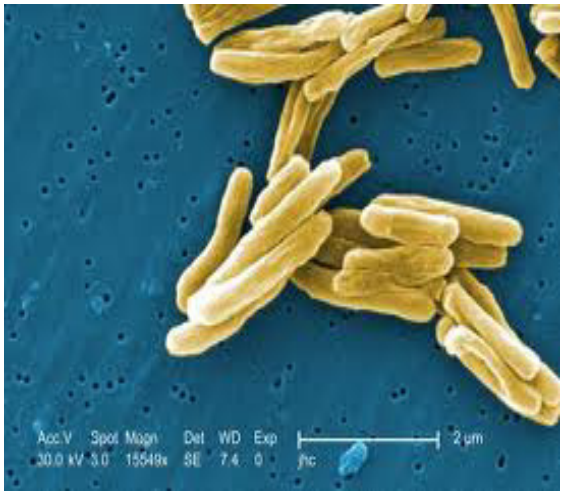
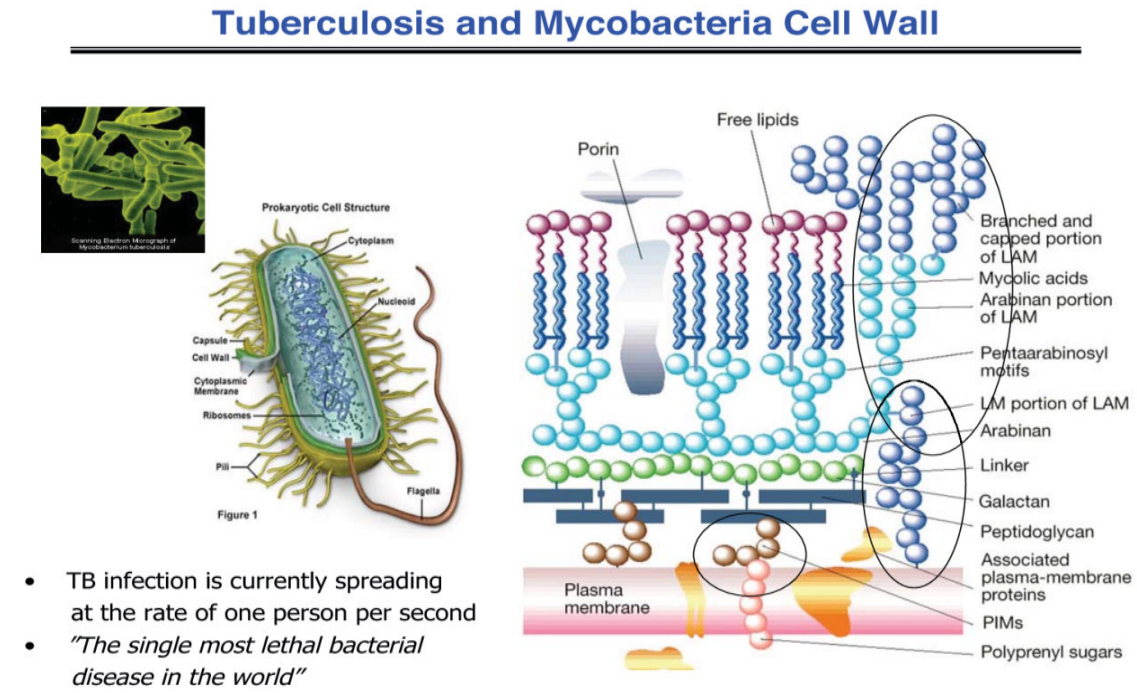


Figure - 2



H37Rv cell line:

Mycobacterium tuberculosis is a highly successful pathogens and it's successfully relies on its ability to utilize macrophages for its replication and, more importantly, the macrophages should remain viable to host the mycobacterium. Despite the fact that these phagocytes are usually very effective in internalizing and clearing most of the bacteria, Mycobacterium tuberculosis H37Rv has evolved a number of very effective survival strategies, including

a) The inhibition of phagosome-lysosome fusion.
b) The inhibition of phogosome acidification
c) The recruitment and retention of tryptophan aspartate containing coat protein on phagosomes to prevent their delivery to the lysosomes.
d) The expression of members of the host induced repetitative glycine rich protein family of proteins.

However, the mechanisms by which Mycobacterium tuberculosis H37Rv enters the host cell, circumvents host defenses and spreads to neighboring cell are not completely understood.[50]

TUBERCULOSIS:

Tuberculosis (TB) is a contagious disease and is at least as old as mankind caused by mycobacteria, mainly mycobacterium tuberculosis. However, still TB continues to be the second largest killer of adults worldwide, the being HIV. Mycobacteria, is a slow-growing bacteria that thrive in areas of the body that are rich in blood and oxygen. TB in the lungs is easily spread to other people through coughing or laughing. Tuberculosis is a curable and controllable disease.

Only people who are sick with TB in their lungs are infectious. Left untreated, each person with active TB disease will infect on average between 10 and 15 people every year. But people infected with TB bacilli will not necessarily become sick with the disease. The immune system "walls off" the TB bacilli which can lie dormant for years. When someone's immune system is weakened, the chances of becoming sick are greater.[51]

INCIDENCE OF TB:

The global burden of tuberculosis (TB) is staggering. An estimated 8.3 million people developed active TB and nearly 2 million people died of TB in 2000 alone.

Tuberculosis (TB) kills two million people a year-one person every 15 seconds. The global incidence of TB disease is rising by 1% annually. Most importantly, the emergence of multidrug-resistant TB (MDR-TB) is posing a new challenge. Approximately 44,600 new MDR-TB patients are being reported in India every year. Globally, India and china are responsible for about 50 percent of the MDR-TB cases.

An estimated 1 billion people will be newly infected between 2000 and 2020, 200 million will fall ill and 35 million will die. TB is a leading cause of death among living with HIV/AIDS.[52]

Risk factors for TB include the following:

- HIV infection
- Low socioeconomic status
- Alcoholism
- Homelessness
- Crowded living conditions

- Diseases that weaken the immune system
- Migration from a country with a high number of cases and health-care workers[53]

Symptoms of Tuberculosis:

1. Poor appetite
2. Night sweets
3. Muscle weakness
4. Fever
5. Dry cough
6. Weight loss

TRANSMISSION:

Spreads through the air when a person with active TB

- Coughs
- Speaks
- Laughs
- Sneezes
- Sings

Another person breathes in the bacteria and become infected

Some signs of TB disease are:

- A bad cough that lasts 3 weeks or longer
- Pain in the chest

- Coughing up blood or phlegm from deep inside the lungs
- Weakness or feeling very tired
- Losing weight very kind
- Having no appetite
- Chills and fever
- Sweating at night or when you are sleeping[54]

CURRENT THERAPY [55]

Drugs used in the treatment of tuberculosis can be divided into two groups.

First-line:

The first line agents include isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), streptomycin and EMB. These agents combine the greatest level efficacy with an acceptable degree of toxicity.

Second-line

These agents include ofloxacin, ciprofloxacin, ethionamide, para amino salicylic acid, cycloserin, amikacin, kanamycin and capreomycin. Because of microbial resistance or patient related factors such as HIV infection, it may be necessary to resort to second line drugs in addition.

Depending upon Chemical Structure Classification:-

- Aminosalicylates and derivatives: PAS, Benzoyl PAS
- Heterocyclic amides: Pyrazinamide, Ethionamide, Prothionamide
- Hydrazides and derivatives: Isoniazid (INH), Iproniazid, sulfoniazid

- Thiourea/thiosemicarbazone derivatives: Thioacetazone, etocarlide
- Ethylene diamine derivatives: D-ethambutol
- Rimino Phenazine derivatives: Clofazimine
- Antitubercular antibiotics
    - Aminoglycosides: Streptomycin, Kanamycin, Amikacin
    - Polypeptides: Capreomycin, Viomycin
    - Ansamycin derivatives: Rifamycin B, Rifamycin, Rifabutin
    - Isoxazolidone derivatives: Cycloserine
- Newer Anti-tubercular agents (Investigational Drugs)
    - Fluoroquinolones: Sparfloxacin, Gatifloxacin Moxifloxacin
    - Macrolide antibiotics: Clarithromycin, Azithromycin
    - Nitroimidazopyrans: PA-824

Directly observed treatment-short course therapy (DOTS)

The magnitude of human suffering forced the government of India to launch to – the Revise National Tuberculosis Control Program (RNTCP) in 1993. This is an application to India of the WHO recommended, "Directly Observer Treatment – Short Course Therapy (DOTS)". DOTS is a strategy documented to be extra ordinarily effective worldwide in Europe, in the USA and in south East Asia.

CURRENT REGIMEN [56]

The recommended first-line regimen consists of isoniazid (H), rifampin (R), pyrazinamide (Z), and ethambutol (E) (HRZE) for 2 months daily or thrice weekly, followed by 4 months of HR or six months of HE. Totally patient has to take drugs for 6 months

CONCERN OVER CURRENT REGIMEN :[57]

The main problems encountered in implementing these regimens are,

1. Prolonged course of therapy and poor compliance
2. Drug interactions
3. Adverse events

Length of treatment:

6 months long duration of TB regimen is not patient friendly and most of the patients do not complete the treatment. This has led to the emergence of multi-and extensively drug resistant TB strains, known as MDR-TB and XDR-TB, respectively.

- MDR-TB is defined as resistance to both H and R, and may be any number of other anti-TB drugs.

  For H resistance – RZE given for 12 months is recommended.

  For H+ resistance – ZE+S/ Kmc/ Am/ Cpr + Cipro/ Ofl ± Etm could used.

- **XDR-TB** is defined as resistant to H, R, a FQ, one of Kmc/ Am/ Cpr with or without any number of other drugs. The XDR-TB is virtually untreatable; mortality is high, particularly among HIV positive patients.

Drug interactions:

Rifamipicin reduces the concentration of most ARVs, due to enzyme induction when TB regimen used along with ARV drugs to treat HIV-TB co-infection. Due to drug interactions of R with many antiretroviral drugs (ARVs), implementation of DOTS remains problematic in people co-infected with HIV and TB.

Adverse events:

Though TB drugs are generally well tolerated, they can cause significant adverse effects and in some cases are contraindicated. Rifampicin and Isoniazid causes hepatitis and pheripheral neuropathy respectively.

The implementation of TB chemotherapy in the field in the field would be much easier if the duration of therapy could be shortened without sacrificing efficacy, a feat that will require at least one new anti-tuberculosis drug. To address these emerging challenges, WHO has been initiating new drug development ideally to shorten and simplify the duration of TB Treatment and address MDR-TB.

Miladis Isabel Camacho-Pozo *et al.,* (2010) found that antimicrobial activity of extracts of *Tamarindus indica L.* leaves were tested against Bacillus subtilis, Enterococcus faecalis, staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa and Candida albicans. The essential oil exhibited a good antimicrobial spectrum when pure, but its relative low concentrations in common folk preparations do not allow for any good activity in these extracts.[58]

Bibul Biswas *et al.,* (2013) was found that the antimicrobial potential of *Psidium guajava* leaf extracts of different solvent were tested against Escherichia coli, Salmonella enteritidis, Staphylococcus aureus and Bacillus cereus by well-diffusion method employing 50 µL extract per well. On the basis of the present finding, guava leaf extract might be a good candidate in the search for a natural antimicrobial agent.[59]

IE Oboh *et al.,* (2007) was found that the ethonolic extract of *Sida acuta burm.f.* shows significant inhibitory activity against standard strains and clinical isolates of Staphylococcus aureus, Bacillus subtilis and Streptococcus feacalis. The MIC values obtained using the Agar-dilution test ranged from 5.0 mg/ml – 10.0 mg/ml. Neither the concentrated extract nor its dilutions inhibited Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Candida albicans.[60]

Mendoza-Aguilar *et al.,* (2012) found that the use of the Microplate Alamar Blue Assay (MABA) to assess the susceptibility of Mycobacterium tuberculosis to anti-tubercular and other drugs.[61]

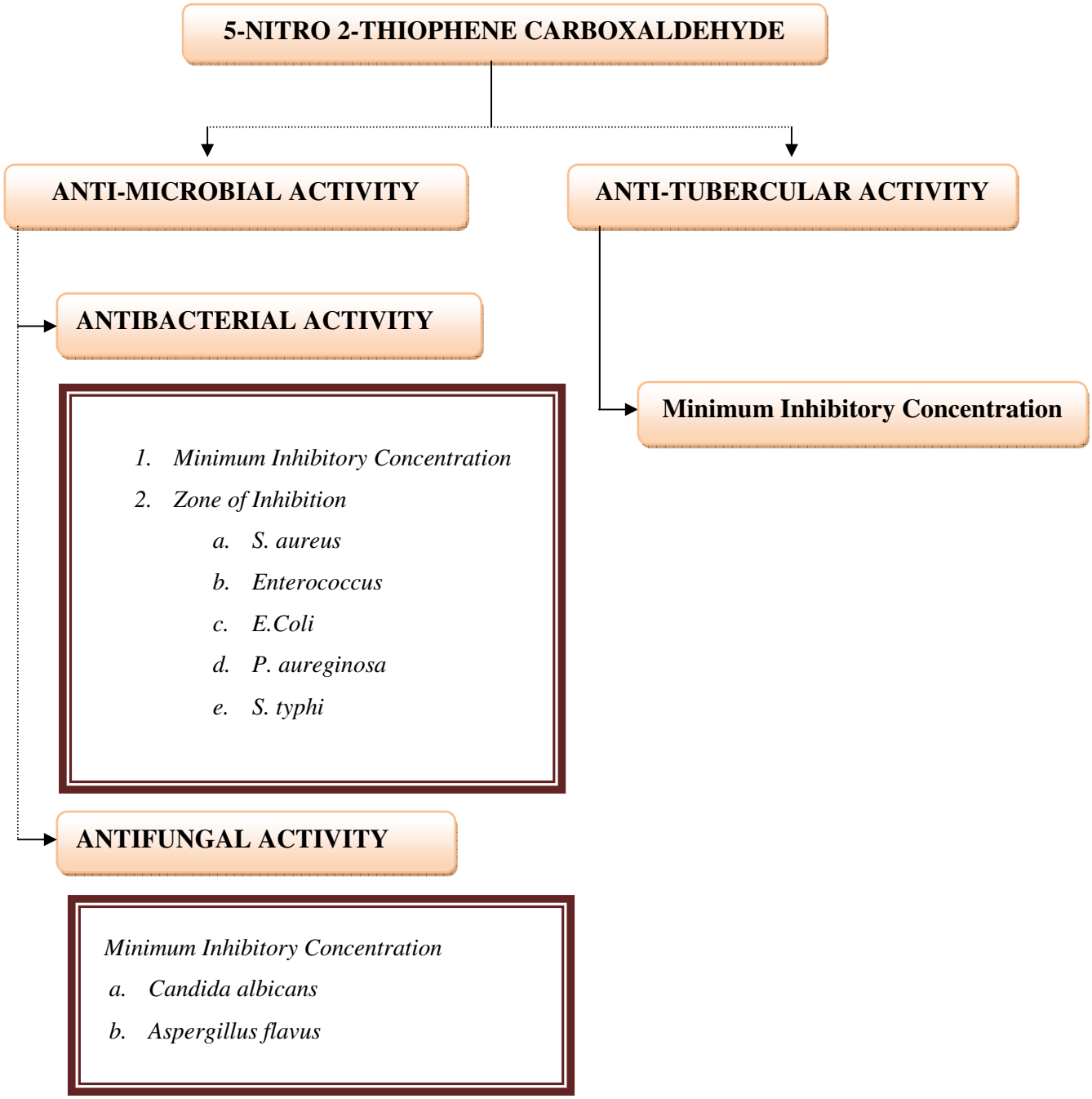
Ibrahim T *et al.,* *(2012)* found that anti-TB activity of Sterculia setigera Del., Leaves (Sterculiaceae). Antitubercular activity of S. setigera was investigated in-vitro on a micro-scale using the Alamar Blue Assay. Three of four successive solvent extractions of the plant leaves extracts inhibited the growth of a virulent strain of Mycobacterium tuberculosis, H37Rv in the concentrations tested (1 – 128

µg/ml). The Minimum Inhibitory Concentration (MIC) determined for the hexane, dichloromethane and ethyl acetate extracts were 84 µg/ml, 62 µg/ml and 128 µg/ml respectively.[62]

Sreenivasulu Munna *et al.,* (2014) found that antitubercular effect of n-Hexane, Chloroform, Ethanol extracts was prepared from whole plant of Actiniopteris radiate Linn was evaluated against Mycobacterium tuberculae using Microplate Alamar Blue Assay (MABA). Minimum Inhibitory Concentration (MIC) was taken to assess antitubercular activity. The result shown that chloroform extract have more significant antitubercular activity as compared to n-Hexane and ethanolic extracts. Pyrazinamide and Streptomycin was taken as standard drugs.[63]

S. Chand Basha *et al.,* (2012) found that antitubercular effect of n-Hexane, Chloroform, Ethanol extracts was prepared from leaves of Rhinacanthus nasutus (k) was evaluated against Mycobacterium tuberculosis using Microplate Alamar Blue Assay (MABA). Minimum Inhibitory Concentration (MIC) was taken to assess antitubercular activity. The result shows that ethanolic extract have more significant antitubercular activity as compared to n-Hexane, chloroform extracts. Pyrazinamide and Streptomycin was taken as standard drugs.[64]

Plan of Work



Standard drug used: Ciprofloxacin, Gentamycin, Amikacin

MATERIALS AND METHODS

COMPOUND NAME: *5-NITRO 2-THIOPHENE CARBOXALDEHYDE*

Product Number: 302295

The compound was purchased from Sigma-Aldrich Pvt Ltd, Bangalore, Karnataka.

ANTIMICROBIAL ACTIVITY:

1. Anti-bacterial activity
2. Anti-fungal activity

ANTIBACTERIAL ACTIVITY:

5-Nitro 3-Thiophene Carboxaldehyde were subjected to antibacterial studies against Gram positive and Gram negative organism.

The organisms used were:

1. Staphylococcus aureus
2. Enterococcus
3. Escherichia coli
4. Peudomonas aureginosa
5. Salmonalla typhi

These organisms were maintained on Nutrient agar slopes in the Institute of Microbiology, MMC, Chennai and the organisms were confirmed by bio-chemical tests. They were stored at 4ºC.

MEDIUM:

Mullar Hinton agar media were obtained from Himedia Laboratory Mumbai – 400086, India.

CHEMICAL AND REAGENTS:

The standard analytical grade reagents

1. Dimethyl sulphoxide (DMSO)
2. Water
3. Ciprofloxacin
4. Gentamycin
5. Amikacin

PREPARATION OF THE BACTERIAL SUSPENSION OF INACULATION :[65]

Few colonies of the pathogenic strains were picked and inoculated into 4ml of peptone water. These tubes were incubated for 2-5 hours to produce a bacterial suspension. The suspension was then diluted if necessary with the saline solution to a density, visually equivalent to that of standard, prepared by adding 0.5ml of 1% barium chloride to 99.5 ml of 1% of sulphuric acid. This suspension was then used for seeding.

PREPARATION OF *5-Nitro 2-Thiophene carboxaldehyde* FOR MICROBIAL ACTIVITY TESTING:

The compound dilution were prepared by dissolving the *5-Nitro 2-Thiophene carboxaldehyde* compound in Dimethyl sulphoxide (DMSO) and make up the standard volume by distilled water, But Ciprofloxacin, Gentamycin and Amikacin are used as a standard drug which are dissolved and diluted by water.

Table – II:

COMPOUND NAME	CONCENTRATION (mcg/ml)	COMPOUND (ml)	AGAR MEDIUM(ml)	TOTAL MEDIUM VOLUME (ml)
5-NITRO 2-THIOPHENE CARBOXALDEHYDE	1 µg/ml	1.2	13.8	15
	2 µg/ml	1.2	13.8	15
	3 µg/ml	1.2	13.8	15
	4 µg/ml	1.2	13.8	15
	5 µg/ml	1.2	13.8	15
	6 µg/ml	1.2	13.8	15
	7 µg/ml	1.2	13.8	15
	8 µg/ml	1.2	13.8	15
	9 µg/ml	1.2	13.8	15
	10 µg/ml	1.2	13.8	15

PREPARATION OF MUELLER HITON AGAR MEDIUM:

Materials required for the preparation of one liter of Mueller Hinton Agar medium,

1. Beef infusion 300g
2. Acid hydrolysate of casein 17.5g
3. Starch 1.5g
4. Agar 17g
5. Distilled water q.s.

The above constituents were weighed and dissolved in water, adjusted the pH 7.3±0.2. The mixture was warmed on water bath till agar dissolved. This was then sterilized in an autoclave at 15 lbs pressure and 121ºC for fifteen minutes and used for sensitivity test.

PREPARATION OF AGAR PLATES:

The plates were prepared using Mueller Hinton Agar medium and different compound (*5-nitro -2-thiophene carboxaldehyde,* ciprofloxacin, gentamycin, amikacin) of various dilutions allowed to solidify and dry. Then a loopful of different bacterial cultures were inoculated at the labeled spots. All the plates were then incubated at 37ºC for 24 hours and results were recorded.

INOCULUM'S PREPARATION:

The inoculum was standardized at $1.5x10^8$ CFU/ml comparing with turbidity standard (0.5 MacFarland tube).

SWAB PREPARATION:

A supply of cotton swabs on wooden applicator sticks was prepared. They were sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat.

ANTIFUNGAL ACTIVITY:

5-Nitro 2-Thiophene carboxaldehyde compound was subjected to antifungal activity studies:

The fungal strains used are,

1. Candida albicans
2. Aspergillus flavus

These organisms are maintained on Sabouraud's dextrose agar media and stored at 4ºC.

MEDIUM:

Sabouraud's dextrose agar media

PREPARATION OF SABOURAUD'S DEXTROSE AGAR MEDIUM:

Materials required for the preparation of 1 liter of sabouraud's dextrose media,

1. Mycological peptone 10g
2. Dextrose 40g
3. Agar 15g
4. Distilled water q.s.
5. Methylene blue dye q.s

The above constituents were weighed and dissolved in water. The mixture was warmed on water bath till agar dissolved and the final pH was adjusted to 5.5±0.2ºC. This was then sterilized in an autoclave at 15 lbs pressure at 121ºC for fifteen minutes, add the required amount of methylene blue dye, shake well and used for the sensitivity test.

The dilutions were prepared by dissolving the *5-Nitro 2-Thiophene carboxaldehyde* compound in Dimethyl sulphoxide and dilution done by using distilled water.

Table – III:

COMPOUND NAME	CONCENTRATION (mcg/ml)	COMPOUND (ml)	SABORAUD'S MEDIUM(ml)	TOTAL MEDIUM VOLUME (ml)
5-NITRO 2-THIOPHENE CARBOXALDEHYDE	1 µg/ml	1.2	3.8	5
	3 µg/ml	1.2	3.8	5
	5 µg/ml	1.2	3.8	5
	7 µg/ml	1.2	3.8	5
	9 µg/ml	1.2	3.8	5
	10 µg/ml	1.2	3.8	5

PREPARATION OF SABORAUD'S PLATES:

The plates were prepared using Saboraud's dextrose agar medium and *5-nitro -2-thiophene carboxaldehyde* of various dilutions allowed to solidify and dry. Then a loopful of different bacterial cultures were inoculated at the labeled spots. All the plates were then incubated at 37ºC for 24 hours-Candida albicans, 48 hours for Aspergillus flavus (here we use the 7 days culture of Aspergillus flavus for excellent growth) and results were recorded.

PROCEDURE FOR ANTIMICROBIAL ACTIVITY STUDIES:

The antimicrobial activity study has been done by 2 methods

1. Minimum Inhibitory Concentration (MIC) - Agar dilution method
2. Zone of Inhibiton by – Agar diffusion method

MINIMUM INHIBITORY CONCENTRATION BY AGAR DILUTION METHOD:

1. The prepared and sterilized medium (15ml for bacterial strain and 5ml for fungal strains) was poured in sterilized petri dishes along with different compound of various dilutions (*5-nitro - 2-thiophene carboxaldehyde*, ciprofloxacin, gentamycin, amikacin) and allowed them to solidify on a plane table.
2. Mark the plate with marker for differentiates various organisms in same plate and then a loopful of different bacterial cultures was inoculated at the corresponding labeled spots.

For bacterial strains:

1. Staphylococcus aureus
2. Enterococcus
3. Escherichia coli
4. Pseudomonas aureginosa
5. Salmonalla typhi

For fungal strains:

- Candida albicans
- Aspergillus flavus

3. The strains inoculated all the plates were incubated at 37ºC for 24 hours all the microbes except Aspergillus flavus because which is incubated for 48 hours at 37ºC.
4. Finally, after the corresponding time is over, the report is recorded.

ZONE OF INHIBITION FOR VARIOUS BACTERIAL STRAINS BY USING AGAR DIFFUSION METHOD:

1. All of the compound (*5-nitro -2-thiophene carboxaldehyde*, ciprofloxacin, gentamycin, amikacin) were dissolved in corresponding solvent and diluted with water for proper concentration.
2. All the compound were taken at the various concentrations (8µg/ml, 16µg/ml, 32µg/ml, 64µg/ml) testing antibacterial activity. The compound diffused into the medium produced a concentration gradient. After the incubation period, the diameter of the zone was measured in millimeter.
3. Following common standard strains were used for screening of antibacterial activity
    1. Staphylococcus aureus
    2. Enterococcus
    3. Escherichia coli
    4. Pseudomonas aureginosa
    5. Salmonalla typhi
4. The plates were inoculated by dipping a sterile swab into into inoculums. Excess inaculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid.
5. The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60ºC after each application. Finally the swab was passed round the edge of the agar surface. The inoculation was dried for a few minutes, at room temperature, with the lid closed.
6. Ditch the bore in agar plate. Add compounds solution in bore.

7. The plates were placed in an incubator at 37 ºC within 30 minutes of preparation of bacteria.
8. After 24 hours incubation, the **diameter of zone was measured and recorded in millimeter.** The measurements were taken with a ruler, from the bottom of the plate without opening the lid.

ANTITUBERCULAR METHOD:

Minimum inhibitory concentration of the Mycobacterium tuberculosis bacterium (H37Rv) is done by Microplate Alamar Blue Assay method (MABA).

SPECIMEN COLLECTION: [66]

A good sputum specimen consists of recently discharged material from the bronchial tree, with minimum amounts of oral or nasal material. Satisfactory quality implies the presence of mucoid or mucopurulent material and is of greater significance than volume. Ideally, a sputum specimen should have a volume of approximately 5ml, although smaller quantities are acceptable if the quality is satisfactory which is collected from NGH institute of Dental science and Research Institute.

Specimens should be transported to the laboratory as soon as possible after collection. If delay is unavoidable, the specimens should be refrigerated to inhibit the growth of unwanted micro-organisms. If refrigeration is not possible and a delay of more than 2 days is anticipated, a suitable preservative i.e. an equal volume of a mixture of 1% Cetyl Pyridinium Choloride (CPC) in 2% sodium chloride solution is recommended.

BIOCHMEMICAL REACTIONS[67]

Several biochemical tests have been described for the identification of mycobacterial species.

i. **Niacin test:**

If niacin has been extracted from the culture, a yellow color will appear in the extract within a few minutes.

ii. **Arylsulphatase test:**

A pink colour indicates a positive reaction.

iii. **Neutral red test:**

This test detects the ability of a strain to bind neutral red in an alkaline buffer solution. Positive tests are obtained with M.tuberculosis, M.bovis, M.avium, M.ulcerans.

iv. **Catalase peroxidase test:**

Effervesence indicates catalase production and browning indicates peroxidise activity.

v. **Amidase test:**

A blue colour indicates a positive test.

vi. **Nitrate reduction test:**

Positive reaction indicates the development of pink or red colour within 30-60 seconds. This test is positive in M.tuberculosis and negative in M.bovis, M.aviuum

CPC containing specimen should be processed as described below:

To the specimen with CPC add 15-20 ml of sterile distilled water (to reduce viscosity)

↓

Tighten cap of the container and mix well by inversion

↓

Centrifuge at 3000g for 15 minutes

↓

Carefully pour off the supernatant

↓

Add approximately 20ml of sterile distilled water to re-suspend the sediment and fill up with sterile distilled water to the brim

↓

Centrifuge again at 3000g for 15 minutes

↓

Decant the supernatant and inoculate deposit on two slopes of LJ medium

↓

Incubate all the LJ media slopes at 37ºC

↓

Check for growth weekly for 8 weeks

MEDIUM:

- Lowenstein-Jensen medium – for maintenance of Mycobacterium tuberculosis culture.
- 7H9 Broth - for growth of Mycobacterium tuberculosis bacterium.

PREPARATION OF LOWENSTEIN-JENSEN MEDIUM:

Lowenstein-Jensen (LJ) medium is most widely used for tuberculosis culture. LJ medium containing glycerol favours the growth of Mycobacterium tuberculosis, while LJ medium without glycerol but containing pyruvate encourages the growth of Mycobacteria bovis.

Ingredients[68]

Mineral salt solution with malachite green

- ✓ Pottasium dihdrogen phosphate anhydrous (KH_2PO_4) 2.4gm
- ✓ Magnesium sulphate anhydrous 0.24gm
- ✓ Magnesium citrate 0.6gm
- ✓ Asparagine 3.6gm
- ✓ Glycerol (reagent grade) 12ml
- ✓ Malachite green, 2% solution 20ml
- ➢ **Malachite green solution 2%**
- ✓ Malachite green dye 2.0g
- ✓ Distilled water 100ml

Dissolve the dye in distilled water completely. Filter and store in refrigerator. Dissolve the ingredients in order in about 300ml distilled water by heating. Add glycerol, malachite green solution and make up 600ml with distilled water. This solution should be sterilized by autoclaving at 121ºC (15 psi) for 30 minutes. Cool to room temperature if required, otherwise stored in the refrigerator.

Homogenized whole egg:

Fresh country hen's eggs those are not more than seven days old, are cleaned by scrubbing thoroughly with a hand brush in water and slope. Let the eggs soak for 30 minutes in soap solution. Rinse eggs thoroughly in running water and soak them in 70% ethanol for 15 minutes. Before handling the clean dry eggs scrub and wash the hands with a disinfectant. Crack the eggs with the edge of the beaker into a sterile flask and beat them in sterile blender for 30 seconds to one minute.

The following ingredients are aseptically pooled in a large, sterile flask and mixed well:

a. Mineral salt solution with malachite green 600ml

b. Homogenized eggs (25-30 eggs, depending on size) 1000ml

After mixing place the bottle in a slanted position in a inspissator and coagulate the medium for 50 minutes at 85ºC and whole media battle should be incubated at 35-37 ºC for 24 hours to check the sterility.

INOCULATION AND INCUBATION PROCEDURES:

INOCULATION PROCEDURES:

Two slopes per specimen are inoculated each with one 5 mm loopful of the centrifuged sediment, distributed over the surface. 100 µl of 0.5 x 106/ml of the Mycobacterium tuberculosis (H37Rv) were cultured in 7H9 medium in the presence of compound. Bottle caps should be tightened to minimize evaporation and drying of media. Care should be taken to avoid using red hot loop and loop should be cooled before inoculation.

INCUBATION PROCEDURE:

All cultures should be incubated at 35-37ºC until growth is observed or discarded as negative after eight weeks. Contaminated slopes are also discarded.

Middlebrook 7H9 Broth Base:

Middlebrook 7H9 Broth Base with added enrichment is recommended for cultivation and sensitivity testing of Mycobacterium tuberculosis.

Composition:

Ingredients	gm/liter
Ammonium sulphate	0.500
Disodium phosphate	2.500
Mono potassium phosphate	1.000
Sodium citrate	0.100
Magnesium sulphate	0.050
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Ferric ammonium citrate	0.040
L-Glutamic acid	0.500
Pyridoxine	0.001
Biotin	0.0005
Final pH (at 25℃)	6.6±0.2

Directions:

Suspend 2.35 grams in 450 ml distilled water. Add either 2ml glycerol or 0.5 gm polysorbate 80. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 10 minutes. Cool to 45 ºC or below and aseptically add contents of 1 vial Middlebrook ADC Growth Supplement (FD019).

PROCEDURE:

a. 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
b. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of *5-Nitro 2-Thiophene carboxaldehyde* and standard drug pyrazinamide were made directly on plate and control plate is made with 7H9 broth and bacterium only.
c. The final drug concentrations tested were 100 to 0.8 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days.
d. After this time, 25 µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 was added to the plate and incubated for 24 hrs.
e. A blue color in the well was interpreted as no bacterial growth, and pink colour was scored as growth.
f. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

RESULTS

Emergence of new infectious diseases such as tuberculosis, bacterial and fungal diseases have stimulated public interest and inspired commitments to control these diseases. Increasing microbial resistance has become a very serious clinical problem for many classes of antibiotics. Therefore, it is an urgent requirement for novel antimicrobial agents to solve the problem of microbial resistance towards conventional antimicrobial agents. Among the various types of heterocyclic compounds, thiophene derivative possesses antibacterial, antifungal and antitubercular activities.

MINIMUM INHIBITORY CONCENTRATION FOR BACTERIAL STRAINS:

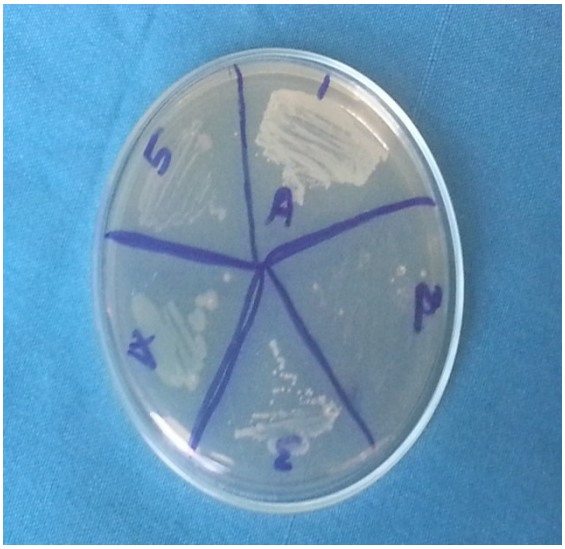
***5-NITRO-2-THIOPHENE CARBOXALDEHYDE*: Table – IV:**

Drug dilution in mcg/ml	ORGANISM				
	GRAM POSITIVE		GRAM NEGATIVE		
	Staphylococcus aureus	Enterococcus	E-coli	Pseudomonas aeruginosa	Salmonalla typhi
1 µg/ml	+	+	+	+	+
2 µg/ml	+	+	+	+	+
3 µg/ml	+	+	+	+	+
4 µg/ml	-	+	+	+	+
5 µg/ml	-	+	+	+	+
6 µg/ml	-	-	+	+	+
7 µg/ml	-	-	-	+	-
8 µg/ml	-	-	-	-	-

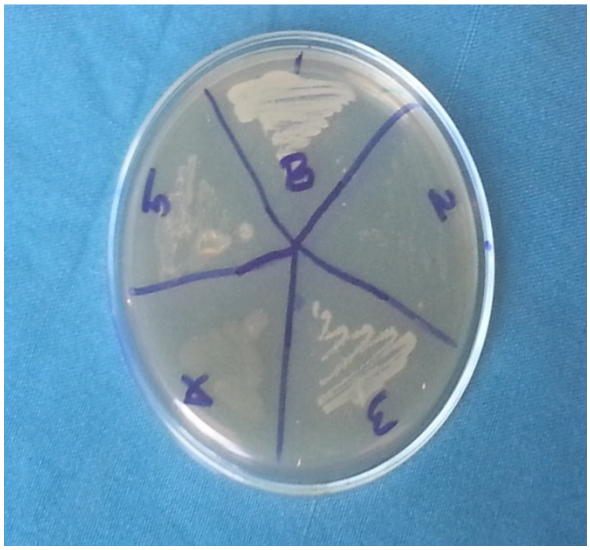
(+) → Bacterial growth

(-) → Inhibition of bacterial growth

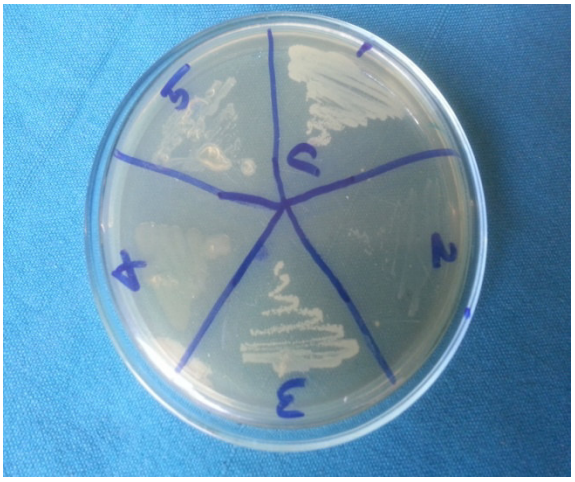
Figure – 3



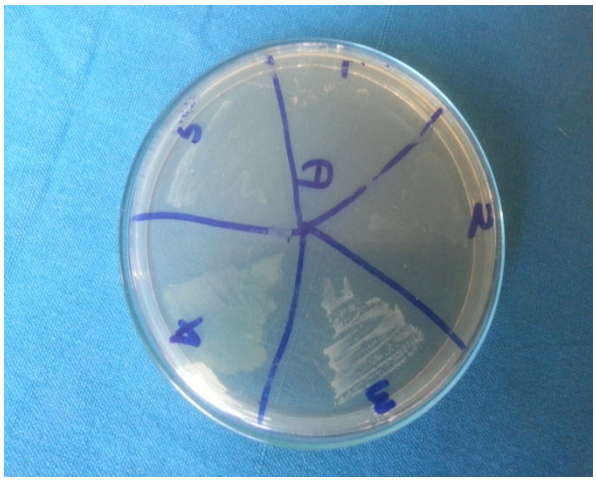
A = 1 µg/ml



B = 2 µg/ml



C = 3 µg/ml

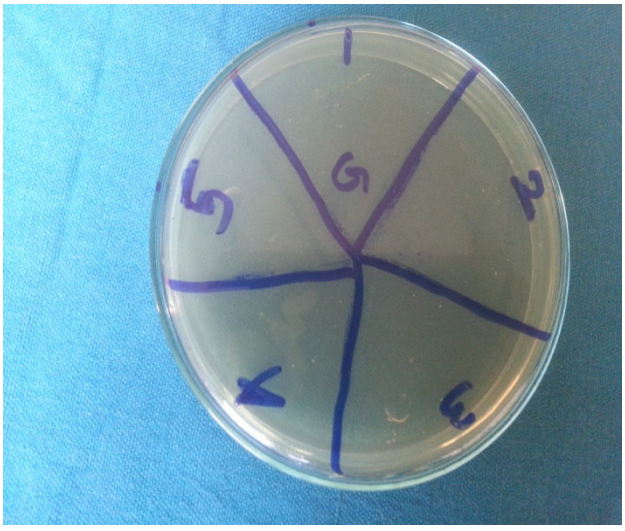


D = 4 µg/ml

Figure – 4:

E = 5 µg/ml

F = 6 µg/ml



G = 7 µg/ml

H = 8 µg/ml

Table – V:

CIPROFLOXACIN:

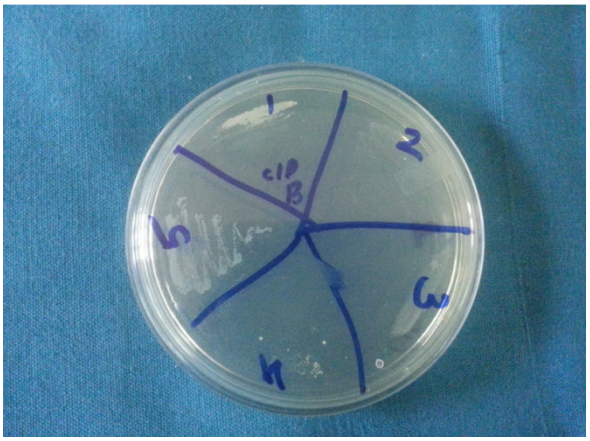
Drug dilution in mcg/ml	ORGANISM				
	GRAM POSITIVE		GRAM NEGATIVE		
	Staphylococcus aureus	Enterococcus	E-coli	Pseudomonas aeruginosa	Salmonalla typhi
1 µg/ml	+	+	+	+	+
2 µg/ml	+	+	+	+	+
4 µg/ml	-	+	-	+	-
8 µg/ml	-	+	-	-	-
16 µg/ml	-	-	-	-	-

(+) → Bacterial growth

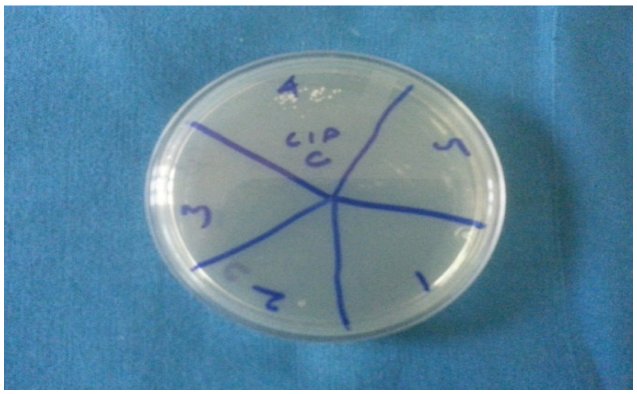
(-) → Inhibition of bacterial growth

Figure -5

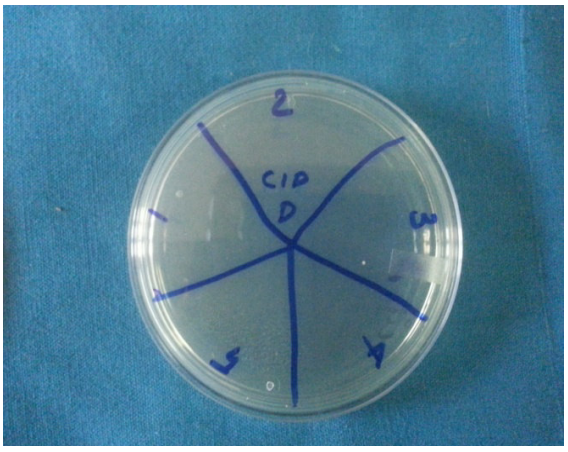
A = 1 µg/ml



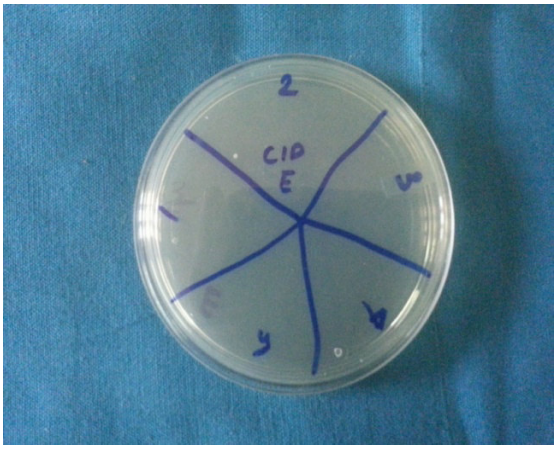
B = 2 µg/ml



C = 4 µg/ml



D = 8 µg/ml



E = 16 µg/ml

CIP → Ciprofloxacin

Table - VI

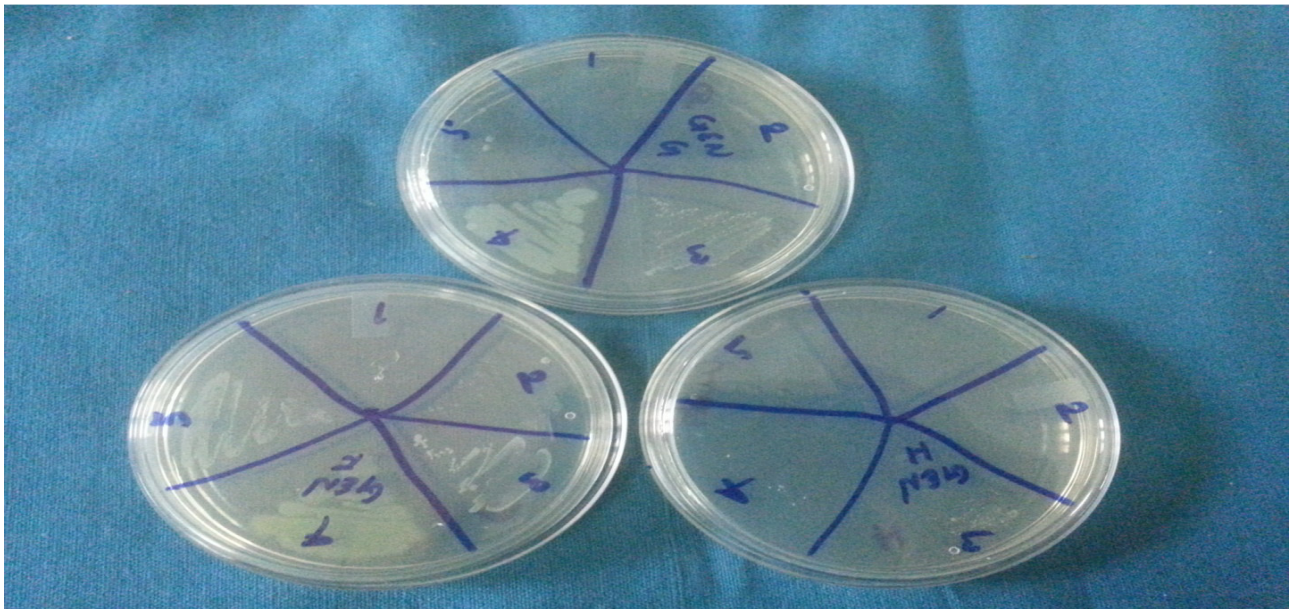
GENTAMYCIN:

Drug dilution in mcg/m	ORGANISM				
	GRAM POSITIVE		GRAM NEGATIVE		
	Staphylococcus aureus	Enterococcus	E-coli	Pseudomonas aeruginosa	Salmonalla typhi
1 µg/ml	+	+	+	+	+
2 µg/ml	-	+	+	+	-
4 µg/ml	-	+	+	-	-
8 µg/ml	-	-	-	-	-
16 µg/ml	-	-	-	-	-
32 µg/ml	-	-	-	-	-

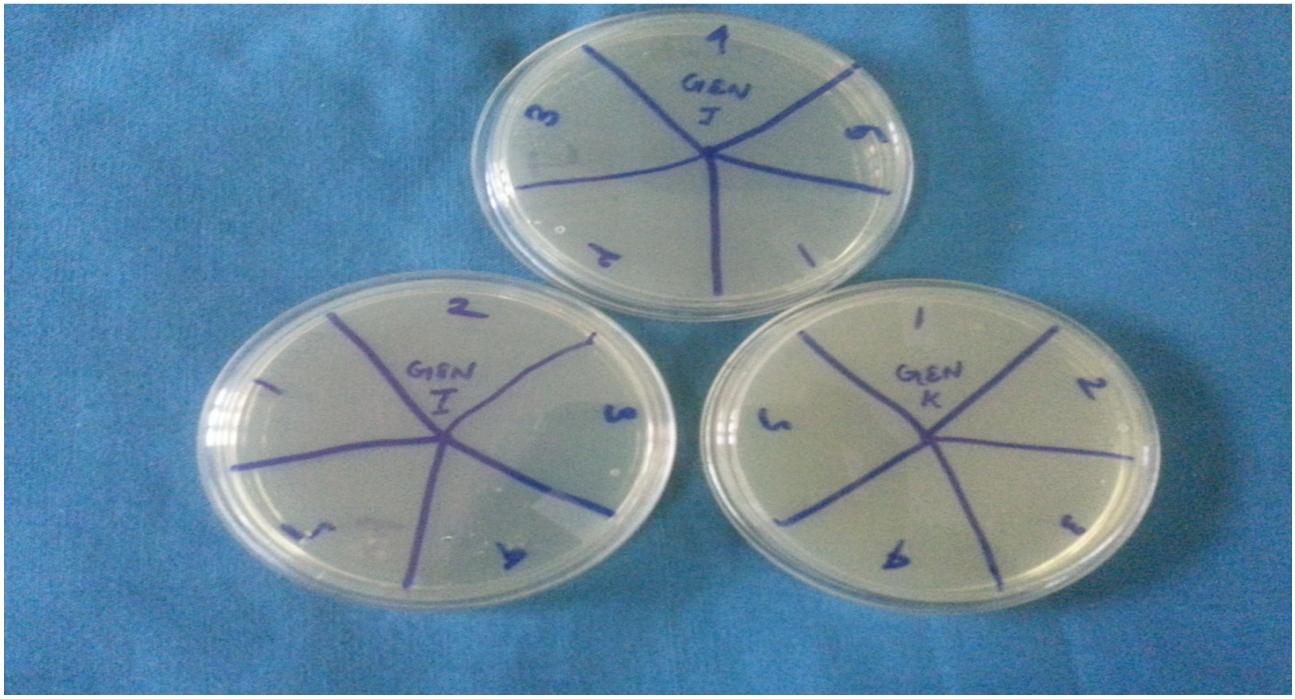
(+) → Bacterial growth

(-) → Inhibition of bacterial growth

Figure - 6



F = 1 µg/ml G = 2 µg/ml H = 4 µg/ml



I = 8 µg/ml J = 16 µg/ml K = 32 µg/ml

GEN → Gentamycin

Table - VII

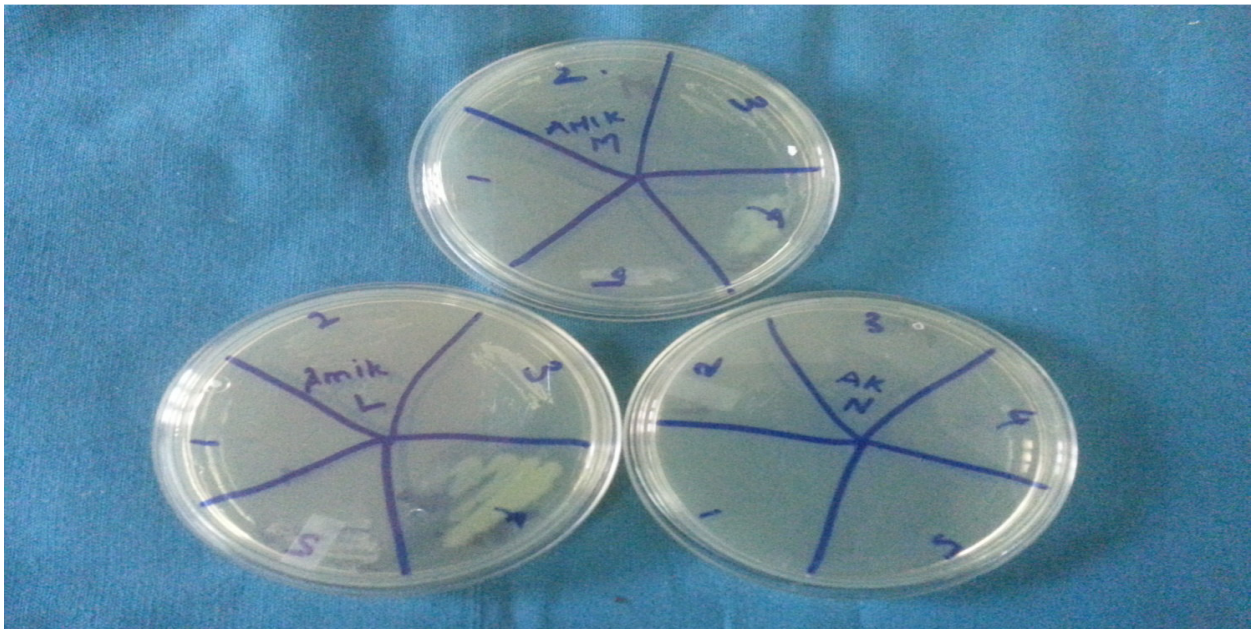
AMIKACIN:

Drug dilution in mcg/m	ORGANISM				
	GRAM POSITIVE		GRAM NEGATIVE		
	Staphylococcus aureus	Enterococcus	E-coli	Pseudomonas aeruginosa	Salmonalla typhi
4 µg/ml	+	+	+	+	+
8 µg/ml	-	+	+	+	+
16 µg/ml	-	+	-	-	-
32 µg/ml	-	-	-	-	-
64 µg/ml	-	-	-	-	-

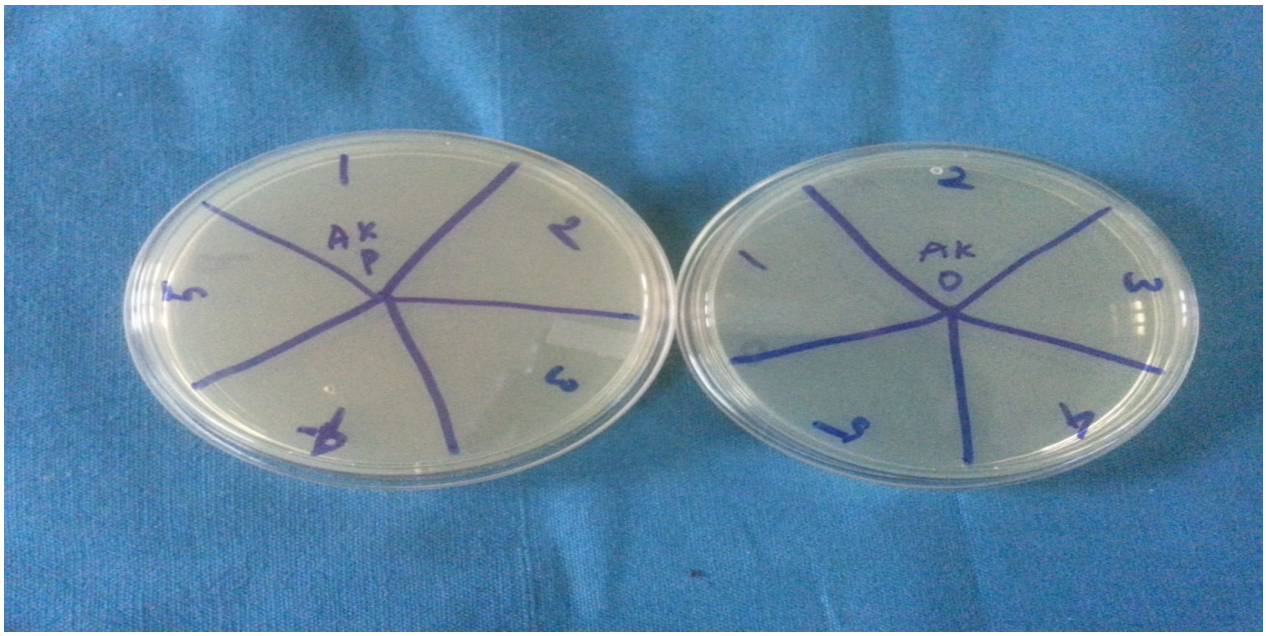
(+) → Bacterial growth

(-) → Inhibition of bacterial growth

Figure - 7



L = 4 µg/ml M = 8 µg/ml N = 16 µg/ml



O = 32 µg/ml P = 64 µg/ml

AMI → Amikacin

Table - VIII

MINIMUM INHIBITORY CONCENTRATION FOR FUNGAL STRAINS:

5-Nitro 2-thiophene carboxaldehyde

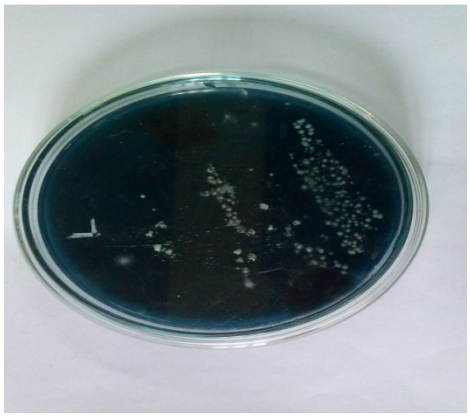
Drug dilution in mcg/m	ORGANISM	
	Candida albicans	**Aspergillus flavus**
1 µg/ml	+	+
3 µg/ml	+	+
5 µg/ml	+	+
7 µg/ml	+	+
9 µg/ml	-	+
10 µg/ml	-	-

(+) → Fungal growth

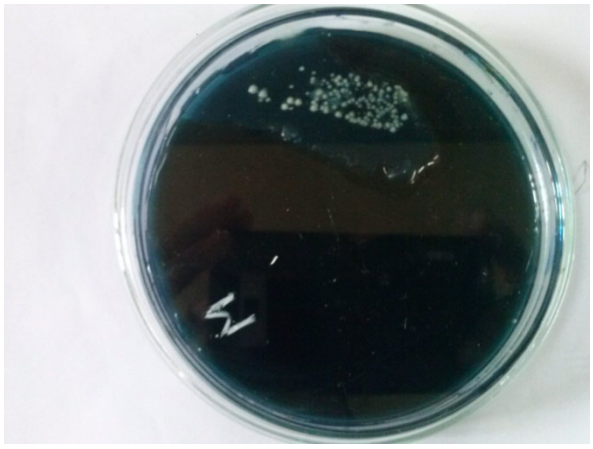
(-) → Inhibition of fungal growth

Figure – 8 Candida albicans

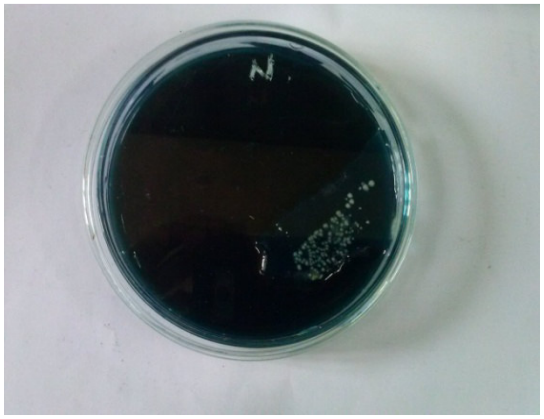
K = 1 µg/ml



L = 3 µg/ml



M = 5 µg/ml



N = 7 µg/ml

O = 9 µg/ml

Figure – 9 Aspergillus flavus

A = 1 µg/ml B = 3 µg/ml C = 5 µg/ml

D = 7 µg/ml E = 9 µg/ml F = 10 µg/ml

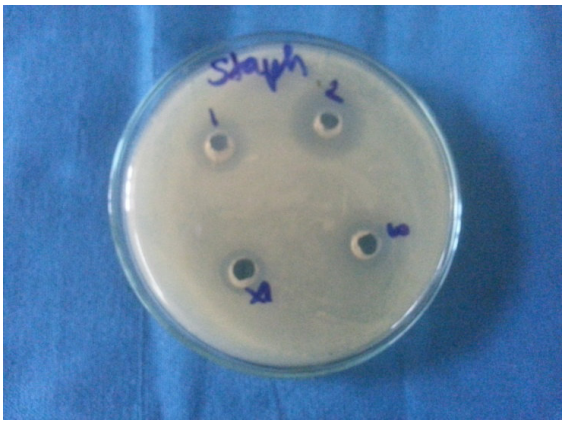
ZONE OF INHIBITION BY AGAR WELL DIFFUSION METHOD:

5-NITRO 2-THIOPHENE CARBOXALDEHYDE:

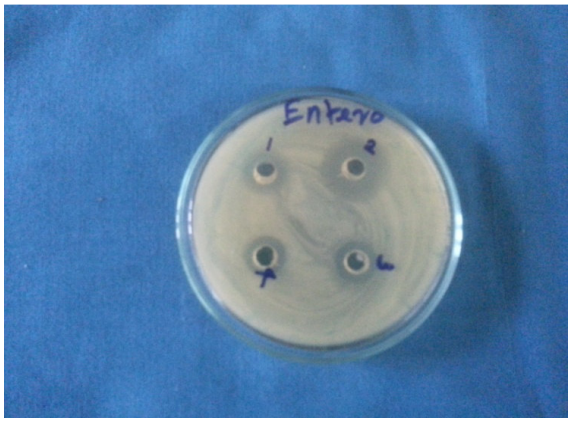
Table - IX

DOSE IN µg/ml	DRUG NAME	ORGANISMS Zone of inhibition in millimeter				
		Staphylococcus aerues	**Enterococcus**	**E-coli**	**Peudomonas aureginosa**	**Salmonalla Typhi**
8 µg/ml	*5- nitro 2-thiophene carboxaldehyde*	13	14	11	12	10
	Ciprofloxacin	20	18	18	22	12
	Gentamycin	15	16	10	11	11
	Amikacin	14	16	10	10	10

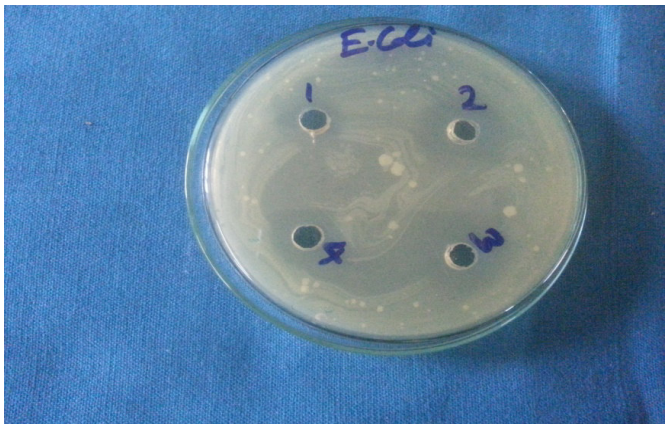
Figure - 11



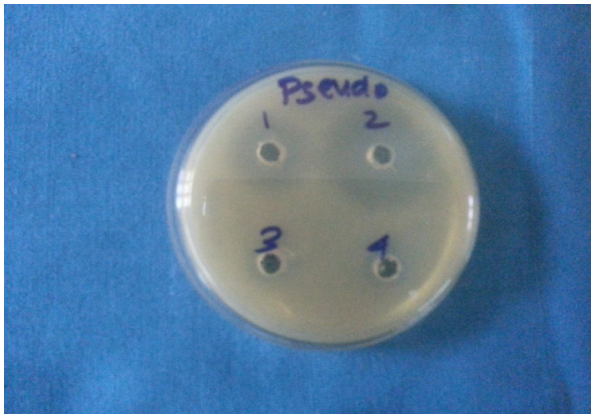
Staphylococcus aerues



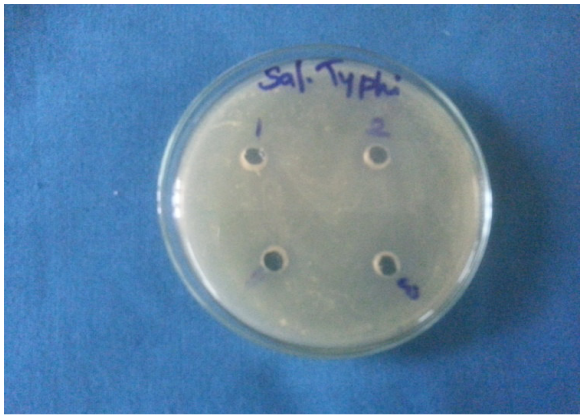
Enterococcus



escherichia coli

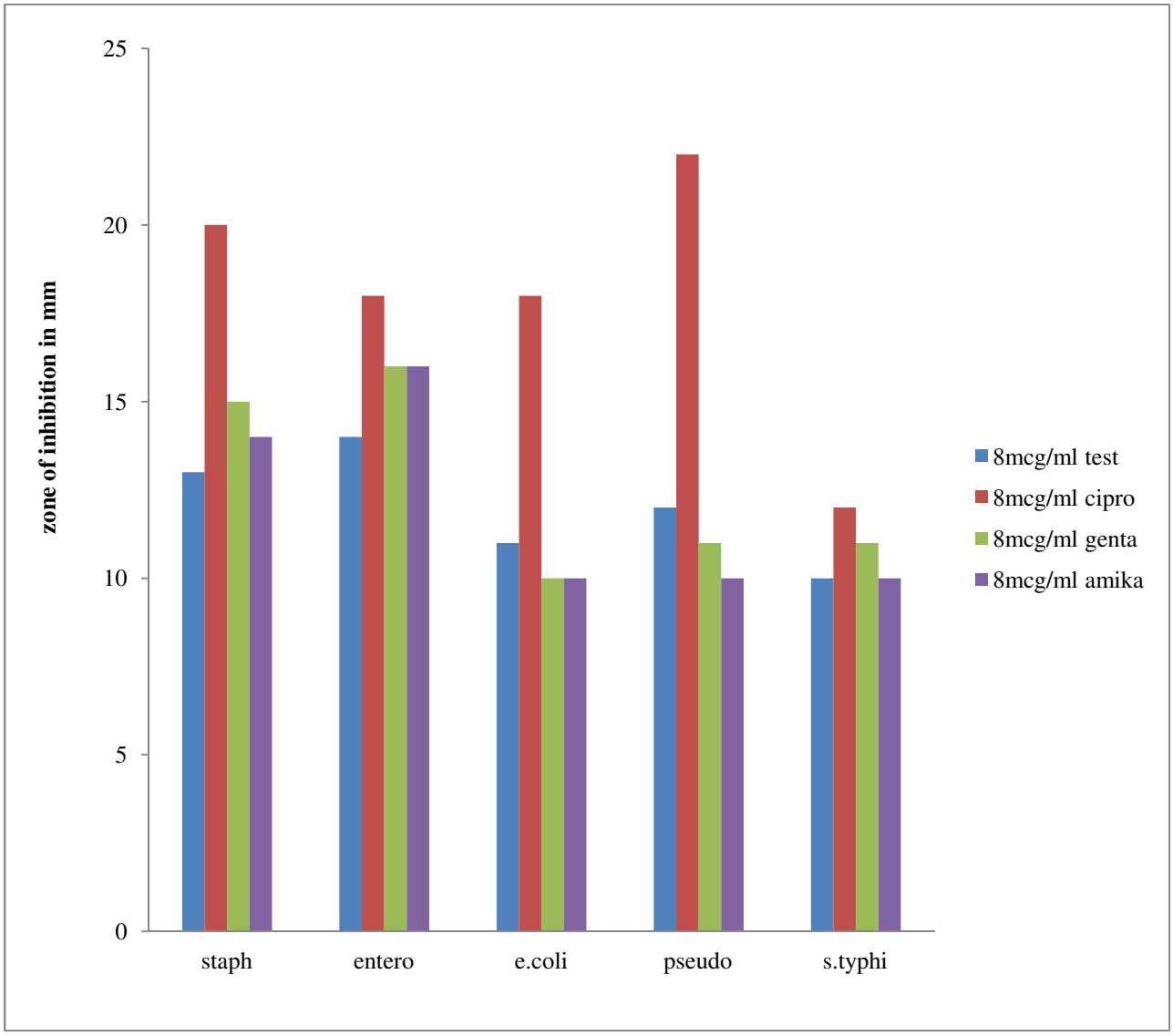


Pseudomonas aureginosa



Salmonalla typhi

Figure - 12



Test drug → *5-Nitro 2-thiophene carboxaldehyde*

Cipro → Ciprofloxacin

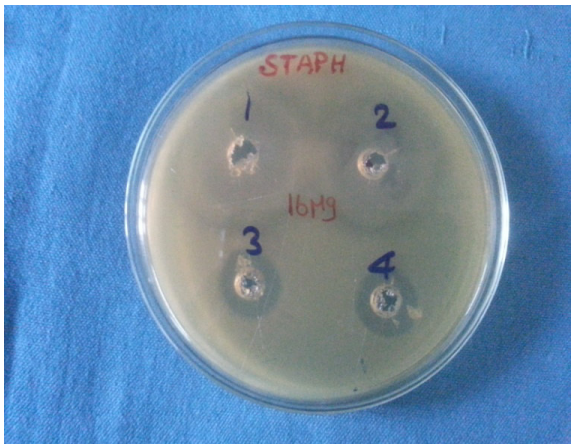
Genta → Gentamycin

Amika → Amikacin

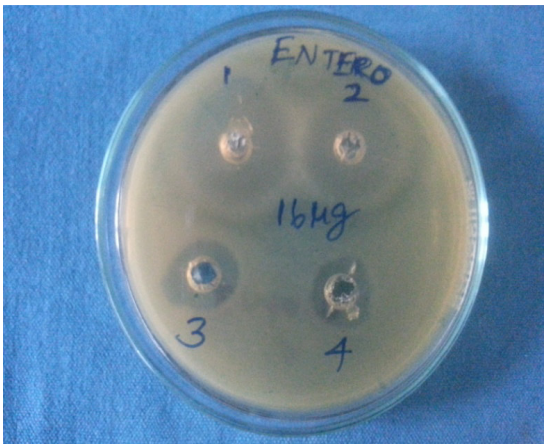
Table - X

DOSE IN µg/ml	DRUG NAME	ORGANISMS (Diameter of the zone in millimeter)				
		Staphylococcus aerues	**Enterococcus**	**E.coli**	**Peudomonas aureginosa**	**Salmonalla Typhi**
16 µg/ml	*5- nitro 2-thiophene carboxaldehyde*	20	18	18	30	17
	Ciprofloxacin	22	21	20	28	25
	Gentamycin	18	16	15	15	16
	Amikacin	19	17	16	15	17

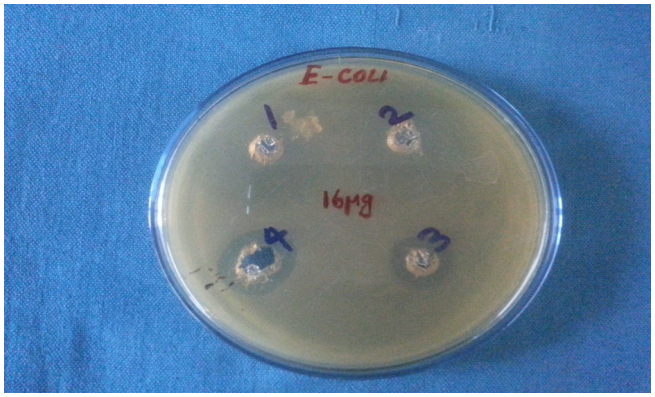
Figure - 13



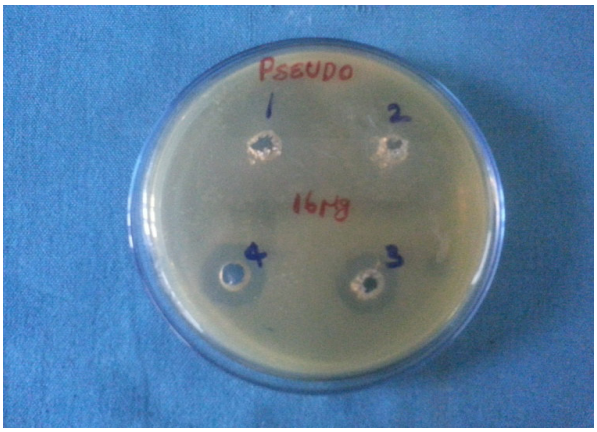
Staphylococcus aureus



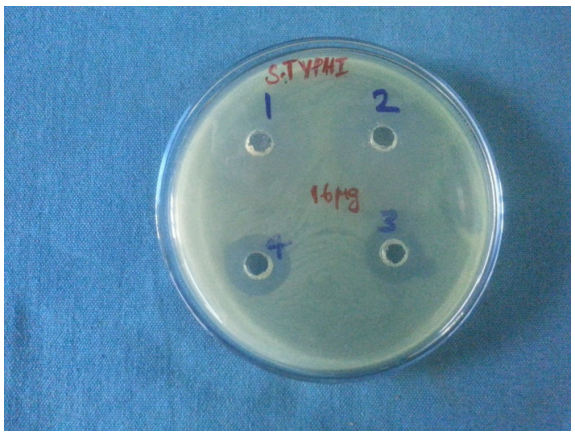
Enterococcus



Escherichia coli

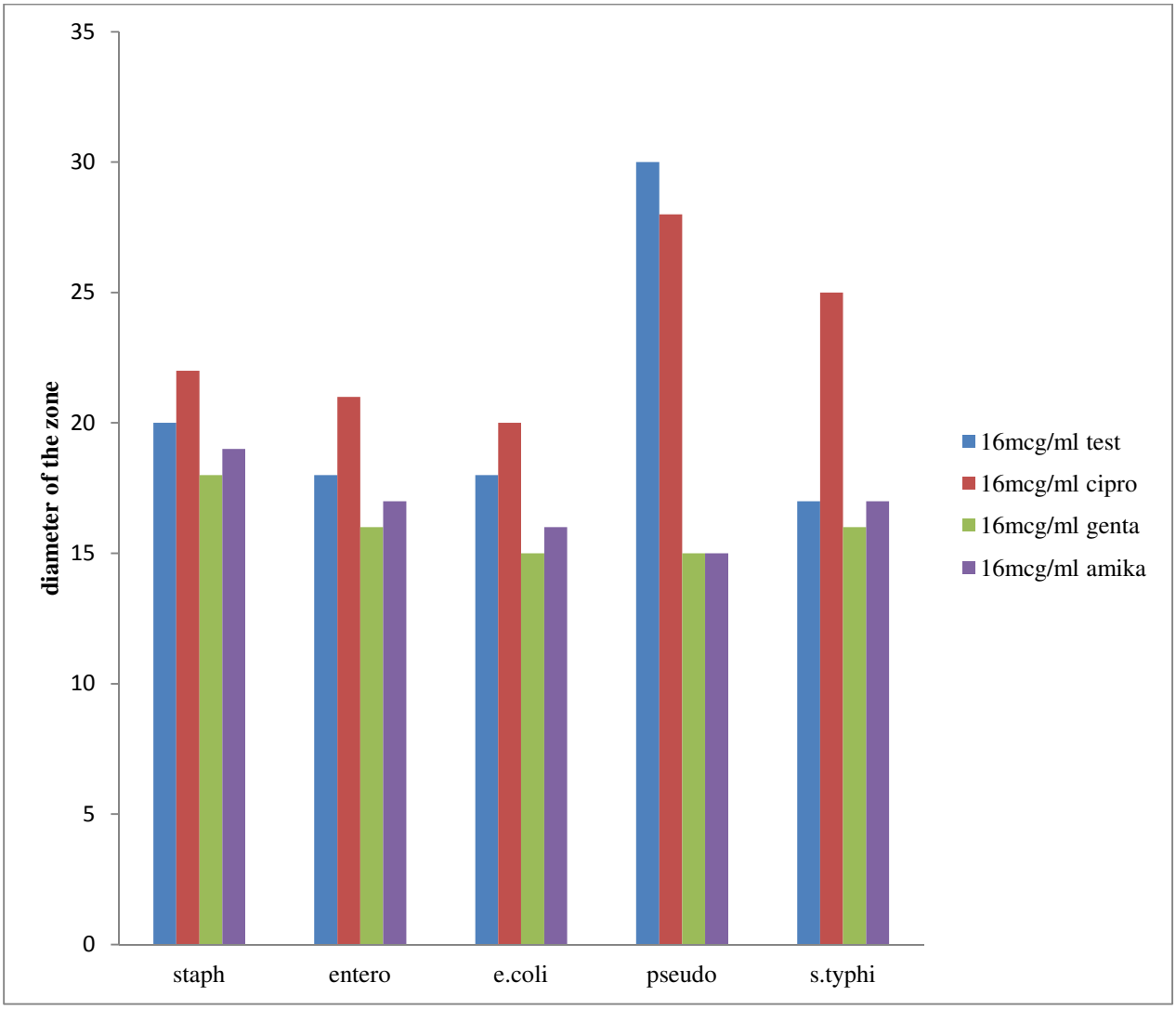


Pseudomonas aureginosa



Salmonalla typhi

Figure -14



Test drug → *5-Nitro 2-thiophene carboxaldehyde*

Cipro → Ciprofloxacin

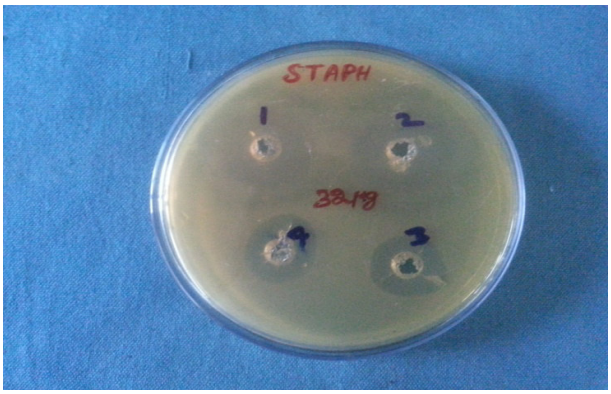
Genta → Gentamycin

Amika → Amikacin

Table - XI

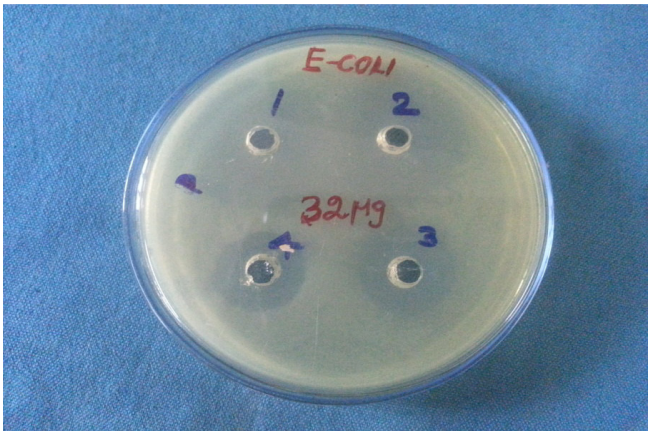
DOSE IN µg/ml	DRUG NAME	ORGANISMS Diameter of the zone in millimeter				
		Staphylococcus aerues	**Enterococcus**	**E-coli**	**Peudomonas aureginosa**	**Salmonalla typhi**
32 µg/ml	*5- nitro 2-thiophene carboxaldehyde*	24	19	19	38	30
	Ciprofloxacin	23	22	21	32	26
	Gentamycin	20	16	16	20	16
	Amikacin	20	17	20	28	17

Figure - 15

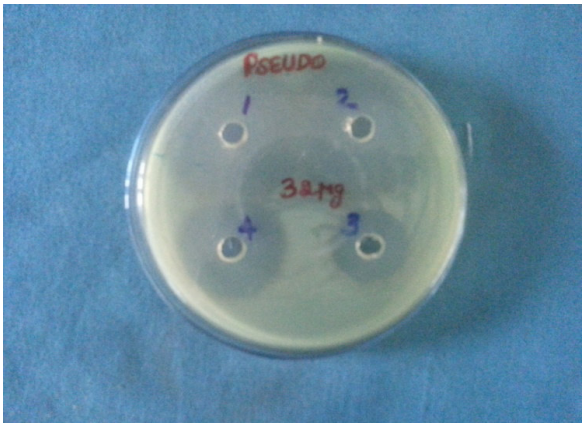


Staphylococcus aureus

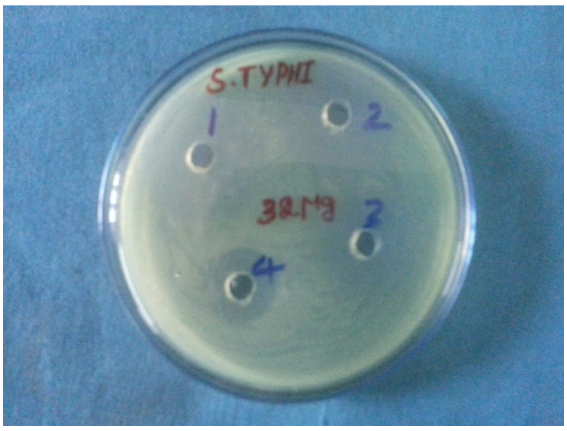
Enterococcus



Escherichia coli

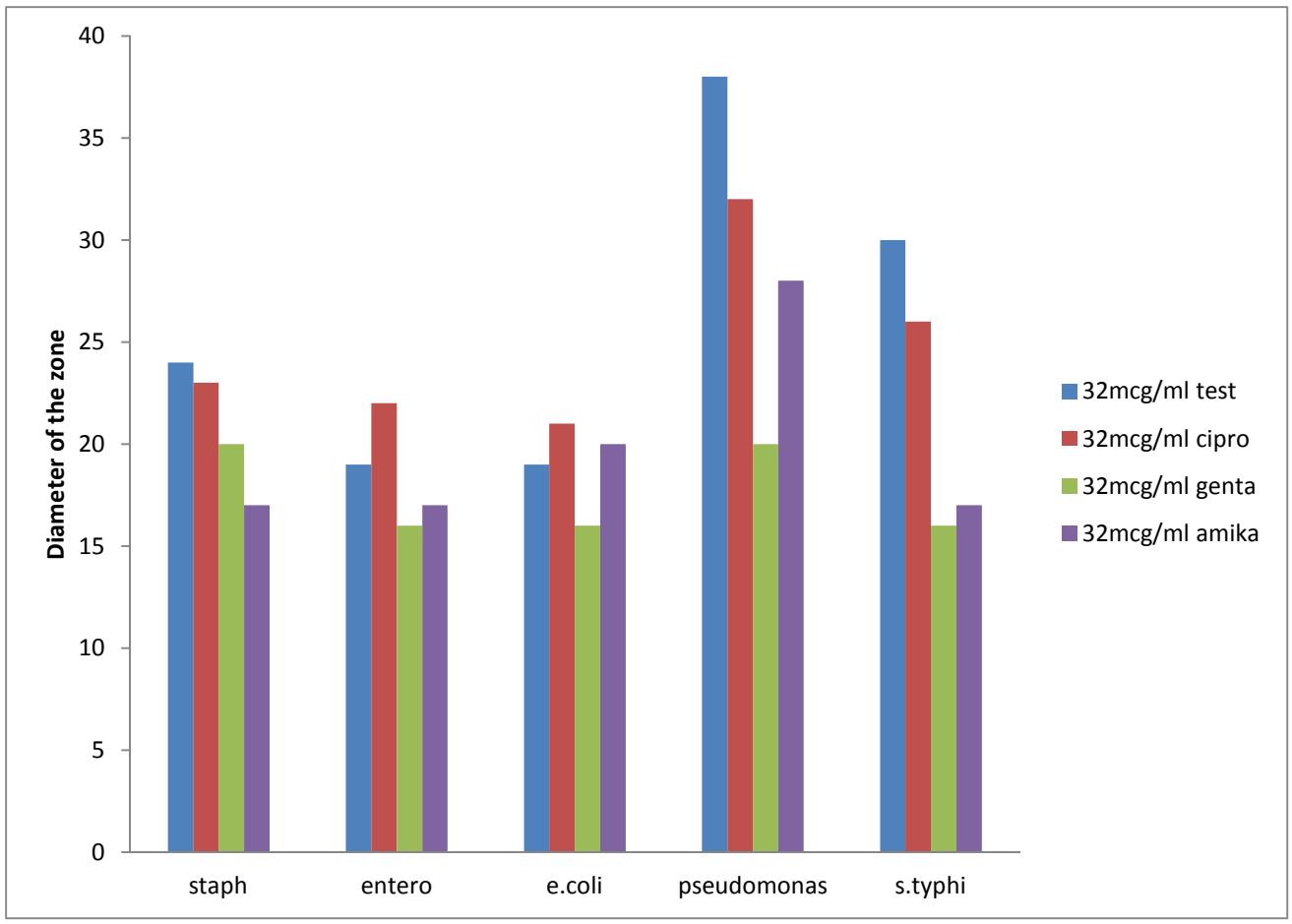


Pseudomonas aureginosa



Salmonalla typhi

Figure – 16



Test drug → *5-Nitro 2-thiophene carboxaldehyde*

Cipro → Ciprofloxacin

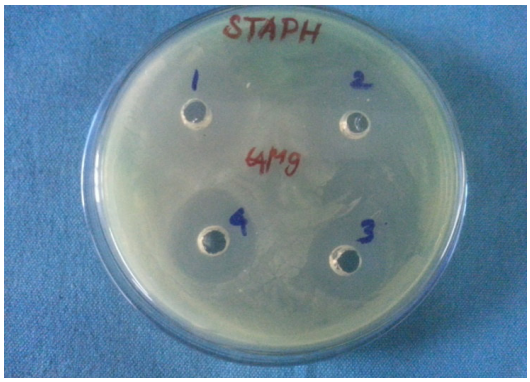
Genta → Gentamycin

Amika → Amikacin

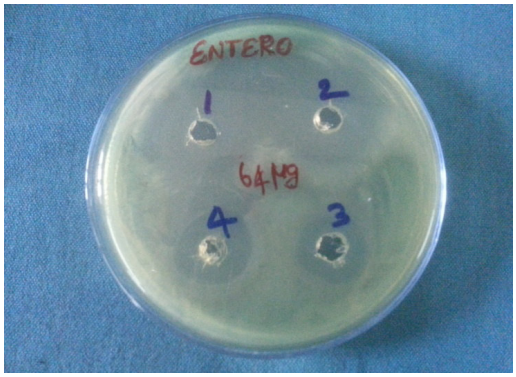
Table - XII

DOSE IN µg/ml	DRUG NAME	ORGANISMS Diameter of the zone in millimeter				
		Staphylococcus aerues	**Enterococcus**	**E-coli**	**Peudomonas aureginosa**	**Salmonalla Typhi**
64 µg/ml	*5- nitro 2-thiophene carboxaldehyde*	25	24	26	40	43
	Ciprofloxacin	25	25	22	35	32
	Gentamycin	20	18	21	20	20
	Amikacin	22	19	25	28	25

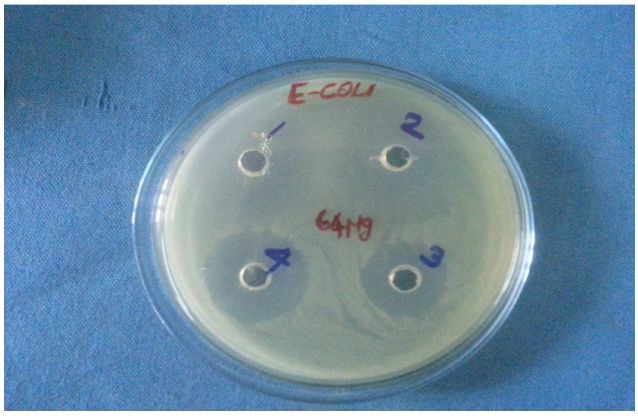
Figure - 17



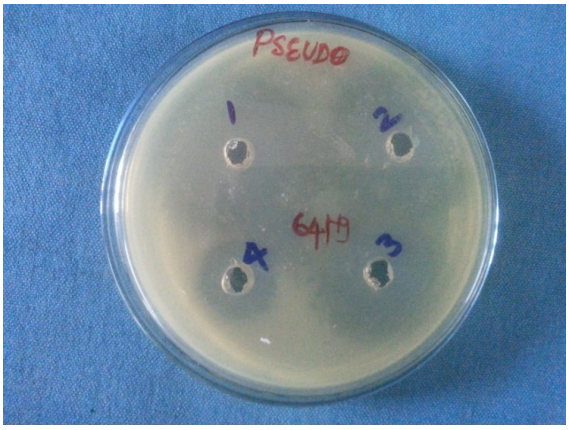
Staphylococcus aureus



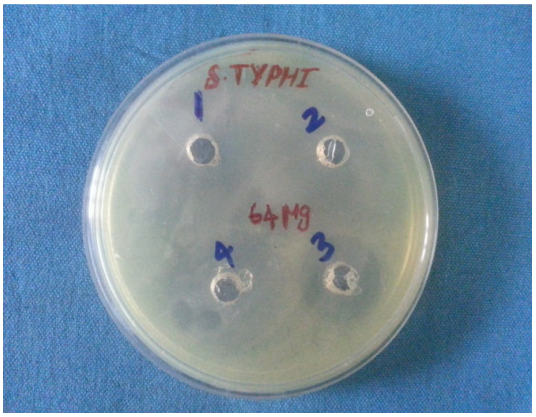
Enterococcus



Escherichia coli

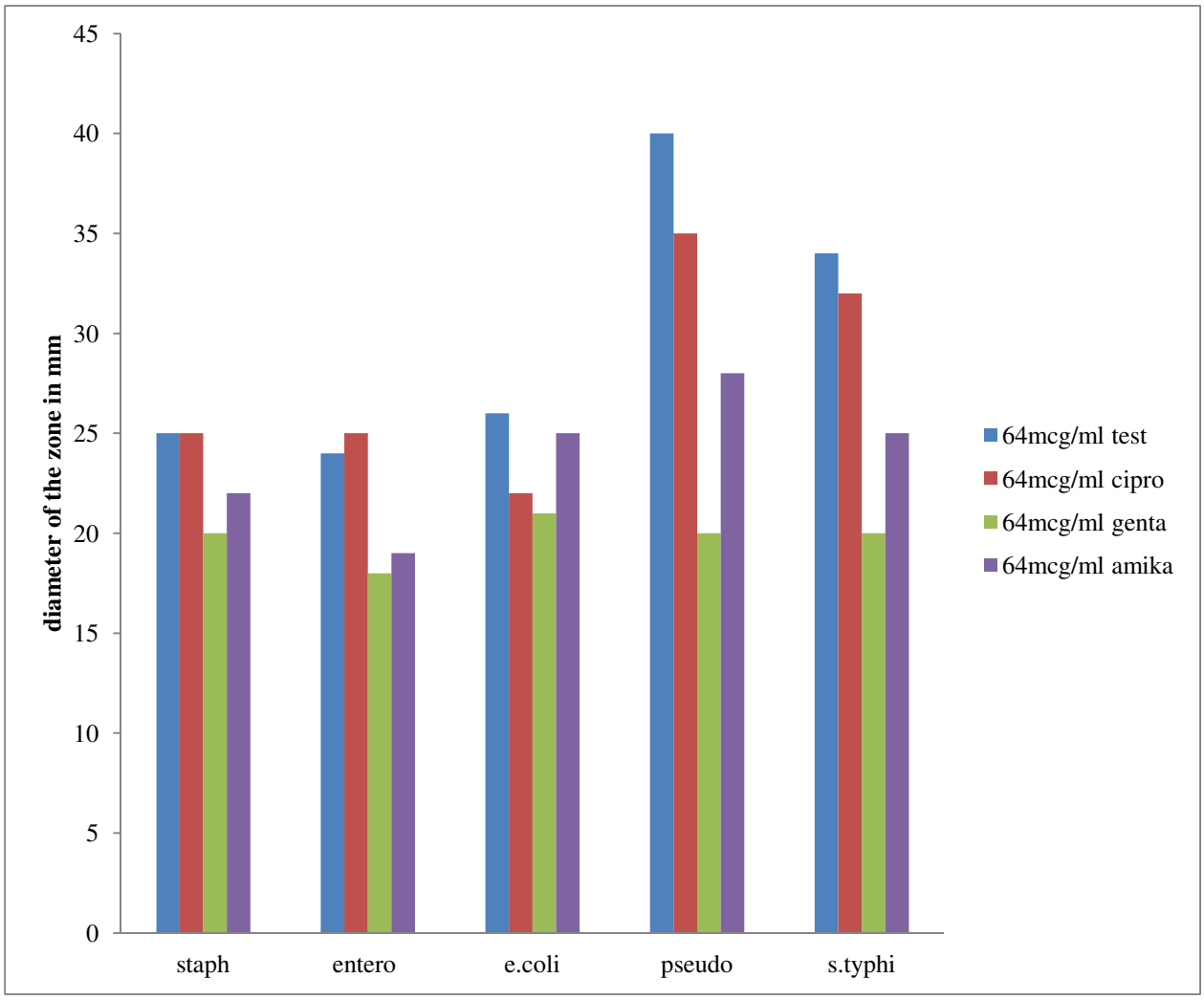


Pseudomonas aureginosa



Salmonalla typhi

Figure - 18



Test drug → *5-Nitro 2-thiophene carboxaldehyde*

Cipro → Ciprofloxacin

Genta → Gentamycin

Amika → Amikacin

ANTI –TUBERCULAR ACTIVITY:-

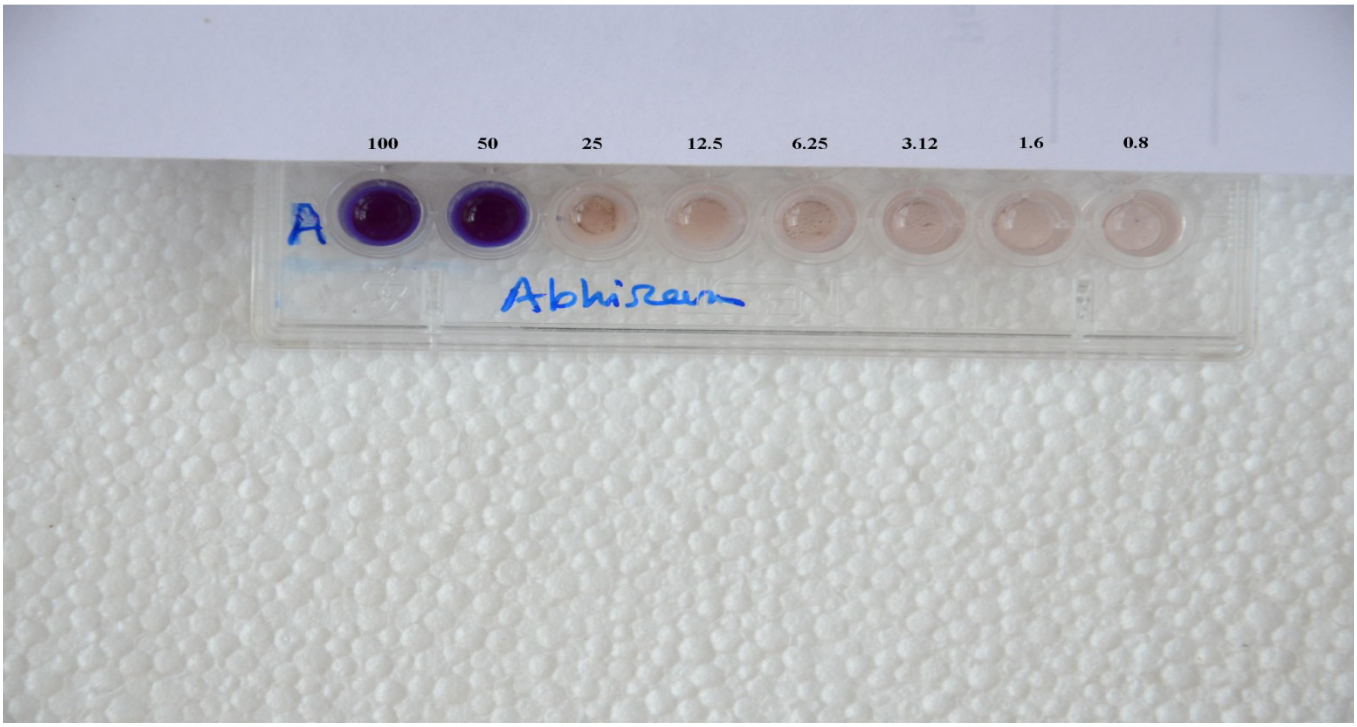
S.NO	SAMPLE NAME	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	*5- Nitro 2–thiophene carboxaldehyde*	S	S	R	R	R	R	R	R

S - Sensitive

R - Resistance

Strain used: Mycobacterium Tuberculosis (H37RV strain)

Figure – 18



Minimum inhibitory concentration of *5-Nitro 2-thiophene carboxaldehyde*

DISCUSSION

The dissertation entitled anti antimicrobial and anti-tubercular activity on ***5-Nitro 2-thiophene carboxaldehyde.*** In the present work, a synthetic drugs ***5-Nitro 2-Thiophene carboxaldehyde*** was selected.

ANTIMIICROBIAL ACTIVITY:

FOR BACTERIAL STRAINS:

MINIMUM INHIBITORY CONCENTRATION:

5-Nitro 2-Thiophene Carboxaldehyde:

- The result shows that the *5-Nitro 2-Thiophene carboxaldehyde* has increased bactericidal activity against gram positive bacteria when compared to gram negative bacteria.
- *5-Nitro 2-Thiophene carboxaldehyde* at the concentration 7mcg/ml seems to be effective against Staphylococcus aureus, Enterococcus, E-coli and Salmonalla typhi but not against pseudomonas aureginosa.
- 8mcg/ml is the critical break point minimum inhibitory concentration for the *5-Nitro 2-Thiophene carboxaldehyde* compound. At this concentration all the tested pathogens were inhibited.

STANDARD DRUGS:

Ciprofloxacin:

- Ciprofloxacin has a better Gram-negative activity than Gram-positive organisms.
- At the concentration of 8mcg/ml, all the tested Gram-negative bacteria and Staphylococcus aureus were inhibited.

- 16mcg/ml is the critical breakpoint of minimum inhibitory concentration for the Ciprofloxacin standard drug. At this concentration all the test drug pathogens were inhibited.

Gentamycin:

- Gentamycin seems to have a good Gram-positive and Gram-Negative activity even at 4mcg/ml
- 8mcg/ml is the critical breakpoint of minimum inhibitory concentration for the Gentamycin drug. At this concentration all the test drug pathogens were inhibited.

Amikacin:

- Amikacin has the best broad spectrum activity at 16mcg/ml.
- 32mcg/ml is the critical breakpoint of minimum inhibitory concentration for the Amikacin drug. At this concentration all the test drug pathogens were inhibited.

FOR FUNGAL STRAINS:

- It has seems to have activity against yeast like fungi when compared to mould.
- Candida albicans was completely inhibited at a *5-Nitro 2-Thiophene carboxaldehyde* concentration of 9mcg/ml. While as Aspergillus flavus was moderately inhibited.
- 10mcg/ml is the critical breakpoint of minimum inhibitory concentration for the *5-Nitro 2-Thiophene carboxaldehyde* compound. At this concentration both yeast like fungi and moulds were completely inhibited.

ZONE OF INHIBITION:

Preliminary antibacterial activity was carried out for *5-Nitro 2-Thiophene carboxaldehyde* compound using Agar well diffusion method against Staphylococcus aureus, Enterococcus, E.coli, Pseudomonas aeruginosa, Salmonalla typhi bacterial strains and by using the standard drugs like Ciprofloxacin, Gentamycin, Amikacin. The compound shows good antibacterial activity on Agar diffusion method by measuring the diameter of the zone. The *5-Nitro 2-Thiophene carboxaldehyde* compound shows antibacterial activity at concentration dependent manner. The diameter of zone of inhibition was more in Pseudomonas aureginosa and Salmonalla typhi at all increasing concentrations (8 µg/ml, 16 µg/ml, 32 µg/ml, 64 µg/ml) than other organisms like Staphylococcus aureus, Enterococcus and E-coli. Order of organisms based on the diameter of the zones: Pseudomonas aureginosa > Salmonalla typhi > Staphylococcus aureus > Enterococcus > E-Coli.

When compared to the standard drugs like Ciprofloxacin, Gentamycin, amikacin, the inhibitory zone diameter at all increasing concentration is relatively equal to that of the ciprofloxacin. So this drug having significant antibacterial activity.

The *5-Nitro 2-Thiophene carboxaldehyde* showed best antimicrobial activity at very low concentration at 8µg/ml. Its potency was greater while comparing to the standard drugs.

Under the standard laboratory conditions, it has been found that 5-*Nitro 2-thiophene carboxaldehyde* at a concentration of 8µg/ml seems to be highly effective against staphylococcus aureus, Enterococcus, E-Coli, Pseudomonas aureginosa and Salmonalla typhi, when tested at a condition of $1.5x10^8$ CFU (Colony forming unit/ml). But under the practical condition *5-Nitro 2-*

thiophene carboxaldehyde would be highly effective against most of the commonly isolated pathogens.

ANTI-TUBERCULAR ACTIVITY:

Invitro anti-tubercular potency of *5-Nitro 2-Thiophene carboxaldehyde* compound was determined by Microplate Alamar Blue Assay (MABA) method. Inhibition of growth of Mycobacterium tuberculosis H37Rv cell lines by MABA method, the compound containing wells after 24 hours of incubation at 37ºC were recorded from the concentration of 100µg/ml to 0.8µg/ml. This compound shows Minimum Inhibitory Concentration (MIC) at **50µg/ml** by preventing the colour change from blue to Pink. Change in the colour from blue to pink was scored as growth. So this compound shows the antitubercular activity.

CONCLUSION

Multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effect on the host including hypersensitivity, immune supervision and allergic reaction. This situation forced scientist to search for new antimicrobial substances to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistances in bacteria of medical importance, there is a constant need for new and effective therapeutic agents.

- *5-Nitro 2-Thiophene carboxaldehyde* compound shows best antibacterial activity.
- *5-Nitro 2-Thiophene carboxaldehyde* compound shows best antifungal activity.
- *5-Nitro 2-Thiophene carboxaldehyde* compound has anti-tubercular activity.

REFERENCES

1. Rang and dale's pharmacology, Churchill livingstone publishers, 6th edition, 2007; p. 661.
2. HL Sharma and KK Sharma, Principles of Pharmacology, Paras Medical Publisher, 2nd edition, 2011; P. 688.
3. Agarwal P, Rai V, Singh R.B. 'Randomized, Placebo-controlled, single blind trial of holy basil leaves in patient with non-insulin-dependent diabetes mellitus'. *International journal of clinical pharmacology and therapeutics,* 1996, Vol 34, P. 406 – 409.
4. Pokhrel NR et al. 'Screening and evaluation of the antimicrobial activity of some medicinal plants of Nepal and isolation of pure antimicrobial compound from Bauhinia Variegate'. *Journal of Ethanopharmacol*, 2000; Vol 89: 61 – 66.
5. Balandrin MF, Klocke JA, Wutule ES, Bollinger WH. 'Natural plant chemicals: Sources of industrial and medicinal materials'. Science 1995; 228: 1154 – 1160.
6. Jones FA. 'Herbs – useful plants, their role in history and today'. *Evr J Gastroentrology Hepatol,* 1996; 8: 1227 – 1231.
7. Rathore M, Sharma N and Sharma K. 'Plant based drugs against tuberculosis infection, novel science'. *International Journal of Medical Science,* 2012; 1: 148 – 154.
8. World Health Organization (WHO). WHO report 2011, tuberculosis prevalence surveys: a handbook: Geneva. http://www.who.int/tb/publications/global_report/2011/pdf/full.pdf. 2010 -2017.
9. Fox W. Whither short-course chemotherapy? Br J Dis Chest, International Journal of Pharmacy, 1981; 75: 331 – 357.

10. World Health Organization (WHO). Treatment of tuberculosis: guidelines for national programmes. Publication no. WHO/CDS/TB/2003.313. Geneva, Switzerland: World Health Organization, 2003; 48.
11. Soundari C and Rajarajan S. 'In vitro screening of lyophilized extracts of Alpinia galangal L. and Oldenlandia umbellate L. for antimycobacterial activity'. *International Journal of Biological and Pharmaceutical Research,* 2013; 4(6): 427 – 432.
12. V. Meyer, Berichte der Deutschen chemischen Gesellschaft, 1883; 16: 1465 -1478.
13. W. C Sumpter, Chemical Reviews, 1944; 34(3): 393 – 434.
14. D. Lednicer, Wiley Interscience, 1999; 14: 187.
15. H. S. Tholappanavara, K.M. Mahadevan, H. N. Harishkumar, P. Basavaraj, N. Gadada. Phosphorous, Sulfar and Silicon and the Related Elements, 2009; 184(7): 1866 – 1879.
16. R. Shakru, N. J. P. Subhashini, K. S. Kumar, Shivaraj, *Journal of Chemical and Pharmaceutical Research,* 2010; 2(1): 38 – 46.
17. R. Kulandasamy, A. A. Vasudeva, J. P. Stables, *Europian Journal of Medicinal Chemistry*, 2009; 44(11): 4376 – 4384.
18. S. Bondock, W. Fadaly, M. A. Metwally, *Europian Journal of Medicinal Chemistry*, 2010; 45(9): 3692 – 3701.
19. W. J. Moree, F. Jovic, T. Coon, J. Yu, B. Li, F. C. Tucci, D. Marinkovic, R. S. Gross, S. Malany, M. J. Bradbury, Bioorganic and Medicinal Chemistry Letters, 2010; 45(7), 2316 – 2320.
20. B. Ramesh, B. Someswara Rao, *E-Journal of Chemistry*, 2010; 7(2): 433-436.
21. M. A. El-Sherbeny, A. A. Abdel-Aziz, M. A. Ahmed, *Europian Journal of Medicinal Chemistry,* 2010; 45(2): 689 – 697.

22. A. E. Rashad, A. H. Shamroukh, R. E. Abdel-Megeid, A. Mostafa, R. El-Shesheny, A. Kandeil, M. A. Ali, K. Banert, *Europian Journal of Medicinal Chemistry*, 2010; 45(11): 5251 – 5257.
23. S. M. Abdel Wahab, N. M. El-Fiki, M. A. Amin, S.F. Mostafa, Bulletin of the Faculty of Pharmacy (Cairo University), 2007; 45 (1): 93 – 104.
24. J. W. Hull, D. R. Romer, T. J. Adaway, D. E. Podhorez, Organic Process Research and Development, 2009; 13 (6): 1125 – 1129.
25. O. Tadaaki, I. Nobuaki, Y. Hiroshi, M. Shin, M. Motoyuki, M. Jun, PCT Int. Appl. WO 2009128537, 2009; pp 46.
26. Dr. Arora, Text book of Microbiology, 2nd ed., New Delhi, CBS publishing company 1999/Book.
27. Robert Cruickshank, Hand book of Bacteriology, Churchill livingstone publishers, 1962; P. 394.
28. K.D. Tripathi, Essential of Medical Pharmacology, Jeepee Brothers, 1994; P. 625.
29. J.W. Churchman, *Journals of Experimental Medicine*, CBS publishing company, 1958; 19: P. 34.
30. A. Albert, *British Journal of Experimental Pathology*, CBS publishing company, 1958; 119: P. 34.
31. L.D. Gebbharadt, J.G. Bachtdd, *Proceeding of the Society for Experimental Biology and Medicine,* 1955; 103: P. 88.
32. E.W. Stearn, A.E. Stearn, *Journal of Bacteriology*, 1924; 9: P. 463 – 479.
33. E. Fisher, R. Muazo, *Journal of Bacteriology,* 1947; 53: P. 381.
34. A. Albert, *British Journal of Experimental Pathology*, 1954; 35: P. 75.

35. A.H. Bakett, *Journal of Pharmacy and Pharmacology*, 1958; 10: P. 160.

36. P.H. Jacobs, Fungal Disease. CBS publishing company, 1997; 1-5.

37. D. Greenwood, Antimicrobial Hemotherapy, 3rd Edition, Jeepee Brothers Medical Publishers, 1995; P. 62.

38. K.D.Tripathi's Essential of Medical Pharmacology, Jeypee Brothers Medical Publishers, 2010; Edition- 6: P. 667 – 727.

39. Arnold LD and Sergio S. 'Microbial drug discovery, 80 years of progress'. *The Journal of Antibiotics,* 2009; 62: 5 -16.

40. Rayn KJ, Ray CG, Sherris Medical Microbiology (4thedition), CBS publishing company, 2004; 4 (3): 43 - 52.

41. Curran JP, Al-Salihi FL. 'Neonatal staphylococcal scaled skin syndrome: massive outbreak due to an unusual phage type'. Pediatrics 66 (2): 285 - 90. PMID 6447271.

42. Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, Rood J. 'Gram-postive pathogens', 1st edition. ASM Press. ISBN 1 – 55581 – 166 - 3.

43. Fisher K, Phillips C. 'The ecology, epidemiology and virulence of Enterococcus', Microbiology 155 (pt 66): 1846 – 57.

44. Lerner CG and Inouye M. 'Pleiotropic changes resulting from depletion of Era, an essential GTP-binding protein in Escherichia coli'. *Molecular Microbiology*, 1991; 5: 951 – 957.

45. Rayn KJ, Ray CG, ed. Sherris Medical Microbiology, 4th edition. McGraw Hill, 2004; PP. 294 – 5.

46. A. Waldman, A Gilhar, L. Duek, I. Berdicevsky. 'Incidence of Candida in psoriasis – a study on the fungal flora of psoriatic patients'. Mycoses, 2003; 44: 77 – 81.

47. Fortwendel JR, Zhao W, Bhabhra R, Park S, Perlin DS, Askew DS and Rhodes JC. ' A fungus-specific ras homolog contributes to the hyphal growth and virulence of Aspergillus flavus'. *Journal of Infectious disease*, 2007; 6: 117.

48. Harsh Mohan, Text book of pathophysiology. 5th edition; 2006; 133.

49. Meena LS, Chopra P, Bedwal RS and Sigh Y. 'Cloning and characterization of GTP-binding proteins of Mycobacterium tuberculosis H37Rv. *Enzyme Microbial Technol*, 2008; 8: 138 – 144.

50. World Health Organization. Globel TB database. Available at: http//www.who.int/globelalatlas/dataQuary/default.asp. Accessed 21 August 2007.

51. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC and Dye C. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 2003; 163: 1009 – 1021.

52. Donald JA. Burger's Medicinal chemistry and drug discovery. Jeypee Brothers Medical Publishers, (5th edition); 816.

53. www.pubmed.com

54. William A. Petri Jr., Drug used in the chemotherapy of Tuberculosis, Mycobacterium avium Complex Disease, and Leprosy. Goodman and Gilmans, The pharmacological basis of therapeutics, 11th edition 2001; 1273.

55. Harries A- a. What are the current recommendations for standard regimens? Chapter 25 in T. Frieden, Tomen's Tuberculosis, 2nd edition, World Health Organization (WHO), 2004; 124 – 128.

56. Harries A- b. What are the most common adverse drug events to first-line tuberculosis drugs, and what is the procedure for reintroduction of drugs? Chapter 31 in T. Frieden, Tomen's Tuberculosis, 2nd edition, World Health Organization (WHO), 2004; 152 – 150.
57. Canetti G, Fox W, Khomenko A, Mahler H.T, Menon N.K, Mitchison D.A, et al., Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. Bull WHO, 1969; 41, 21 – 43.
58. Miladis Isabel Camacho-Pozo, Julio Cesar Escalona-Arranz, Renato Peres-Roses, Imilci Urdaneta-Laffita Jusus Rodriguez-Amado and Irina Licea-Jimenez. 'Antimicrobial activity of extracts from Tamarindus Indica L. leaves'. Pharmacogn Mag. 2010; 6 (23): 242 – 247.
59. Bibul Biswas, Kimberly Rogers, Fredrick McLaughlin, Dwayne Daniels and Anand Yadav. 'Antimicrobial activities of leaf extracts of Guava (Psidium guajava L.) on two gram-negative and gram-positive bacteria'. International Journal of Microbiology, 2013; 10 (4): 35 -43.
60. IE Oboh, JO Akerele and O Obasuyi. 'Antimicrobial activity of ethanol extract of the aerial parts of Sida acuta burm.f.(malvaceae)'. Tropical Journal of Pharmaceutical Research, 2007; 6 (4): 809 – 813.
61. Mendoza-Aguilar M, Almaguer-Villagran L, Jimenez-Arellanes A, Arce-Paredes P, Cid-Gutierrez JL, Rojas-Espinosa O. 'The use of the Microplate Alamar Blue Assay (MABA) to assess the susceptibility of Mycobacterium tuberculosis to anti-tubercular and other drugs'. J. Infect Chemother, 2012; 18 (5):652-61.

62. Ibrahim T. Babalola, Esther A. Adelakun, Yuehong Wang Francis O. Shode. 'Anti-TB activity of Sterculia setigera Del., leaves (Sterculiaceae)'. Journal of Pharmacognosy and Phytochemistry, 2012; 1 (2): 17 – 23.

63. Sreenivasulu Munna, S. Chand Basha, P. Ramalingewara Reddy, N. Pramod, Y. Pradeep Kumar, G. Mahaboob Basha. 'Antitubercular activity of Actinopteris radiate Linn'. Journal of Globel Trends in Pharmaceutical Sciences, 2014; 5(1): 1443 – 1445.

64. S. Chand Basha, Sreenivasulu. M, P. Ramalingeswara Reddy. 'Antitubercular activity of Leaves of Rhinacanthus nasutus (L.)'. Journal of Global Trends in Pharmaceutical Sciences, 2013; 3 (2): 608 – 611.

65. Ahmad N, Fazal H, Abbasi B.H, Farooq S, Ali M and Khan M.A. Biological role of P.nigram L. (Black pepper): A Review. *Asian Pacific Journal of Tropical Biomedicine*, 1 – 10.

66. P. Chakarabarthy's Textbook of Microbiology Published by New Central Book Agency (P)Ltd, 1995; 1st edition: 323 – 327, 225 – 243.

67. PAHO/WHO Advisory committee on Tuberculosis Bacteriology. Manuals of technical standards and procedures for tuberculosis bacterialogy. Part III Sensitivity of M. tuberculosis to anti-TB drugs. PAHO, Marlinez, Argentina, 1998.

68. Ananthanarayan and Paniiker's Text book of Microbiology (8th edition), Jeypee Brothers Medical Publishers, 2010; 4 (5): 85 - 92.